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A ONE-TIME SCREENING OF NEWCASTLE DISEASE HI ANTIBODIES BEFORE SLAUGHTER AS AN INDICATOR OF VACCINE PERFORMANCE IN BROILER CHICKENS

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SUMMARY

Seven poultry farms participated in a study to determine the vaccine performance in broilers vaccinated with Newcastle disease (ND) vaccines following each of the farms own vaccination programmes and procedures. It is foreseen that knowledge of the antibody titres attained by the birds, will help poultry farmers or veterinarians to reflect on all routine procedures taken in their farms in ensuring that the vaccines delivered performed accordingly. The HI antibody titres of a batch of broilers before slaughter may provide these farmers a guide to assist them with future vaccination strategies for their farms. In this preliminary study, just before marketing, at the age of 27-37 days old, 15-20 broilers were submitted for post vaccination HI antibody determination and protection study. The HI-GMT of antibodies of broilers at approximately one month post-vaccination (pre-challenged) titres for five of the farms were below standard, ranging from as low as 1.83 to 4.70 and is associated with its low level of protection afforded i.e. as low as 38-76 %. For three farms, The HI-GMT antibodies were 115.36 and 78.8 and 6.8 and the protection afforded were 100%, 95% and 85% respectively. The lowest percentage of protection afforded were 38% and 44% in chickens vaccinated with killed vaccines at 3 days old. For the seven farms, at total of 122 broilers were screened and challenged, and out of these, 44 birds showed HI titres of <2. Out of these 44 birds, 41% however, survived the challenge.

Keywords: Chicken, Broiler, Newcastle disease, hemagglutination inhibition

INTRODUCTION

Newcastle disease (ND) is one of the major avian diseases worldwide. It is caused by Newcastle disease virus (NDV) or avian Paramyxovirus serotype 1 (APMV-1). NDV causes a devastating disease in birds and remains one of the most important pathogens of poultry (Alexander, 2000). The disease is still a worldwide economic problem resulting in severe losses to the poultry industry. Virulent strains of Newcastle Disease (ND) are enzootic in Malaysia and despite vaccination,ND outbreaks still occurred throughout the country causing damage and serious losses to the farmers. Most of the domestic outbreaks occurred in areas with high density of poultry farms.

As ND is a continuing threat, farmers worldwide are vigilant of this disease and tremendous efforts to control and prevent ND are constantly being carried out through efficient vaccination programs and corresponding serological monitoring (Ricardo et al., 2000). The testing of chickens for NDV antibodies to determine the potency of the vaccine is done at various intervals and in most cases at least 2 weeks after vaccination. Vaccination of chicks against NDV were performed as early as 1-3 days old and in some, vaccination is repeated at the age of 7-10 days, depending on the different vaccination programmes practiced and the types of vaccines used by each poultry farmer. Most of the time, humoral antibody vaccination response will not reached its optimum level after 2 weeks post-vaccination, therefore making it difficult to determine whether the vaccine and vaccination procedures were successful in stimulating the full potency or efficacy potentials.

In this study, we investigated vaccine performance of broilers from seven commercial farms by demonstrating the NDV-HI antibody level attained, in a one-time screening just before marketing, i.e. as early as 27-37 days old, i.e. just before marketing. Knowledge of the antibody titres attained by the birds will help poultry farmers or veterinarians to reflect on all routine procedures taken in their farms in ensuring that the vaccines delivered performed accordingly, and may assist farmers with future vaccination strategies in their farms.

MATERIALS AND METHODS

Participation of farmers

Seven poultry enterprises located in Sitiawan, Perak, with poultry population of 15,000 to 54,000 participated in this study. The broiler birds from the seven farms were of the Cobb breed. Each of the enterprise practiced their own vaccination programmes against NDV and uses either single ND or various combinations (eg + infectious bronchitis vaccine), and types, i.e. either live or killed of the lentogenic or mesogenic vaccine strains. The routes of inoculation depended on the type of vaccines used and are according to the recommendations of the manufacturers. Just a few days before slaughter, i.e. at the age of between 27-37 days old, 15-20 of the broiler chickens from the vaccinated flock were submitted to VRI. VRI diagnostic numbers (077166-077172 and 077361-077367) were assigned for each of the broiler batch submitted from the seven farms.
Protection studies

At VRI, the birds were tagged and sera collected to determine the post-vaccination or pre-challenged titre. The birds were challenged with a dose of $10^{4.3}$ EID$_{50}$ of virulent (v) NDV per bird via the intranasal route. Birds were observed for clinical signs and death for up to 1 week after challenge, where sera was again collected from surviving birds at the end of the week. As controls, unvaccinated SPF chickens of the same age were also challenged intranasally, with the same vNDV, to prove its pathogenicity in chickens. All dead birds were autopsied and observed for NDV lesions in the intestines, cecal tonsils and other organs. Pooled organs of brain, kidney, trachea, lungs and intestines were collected and subjected to viral isolation. ND virus isolated was validated using the HA test as described previously (OIE, 2012).

The HI assay

Sera was obtained at pre and post challenge from all birds and tested by hemagglutination inhibition (HI) assay for HI antibody titers. The HI assay was performed using inactivated NDV antigen according to standard procedures with 4 HAU virus/antigen in 0.025 ml (OIE, 2012). Titers were calculated as the highest reciprocal serum dilution providing complete hemagglutination inhibition. Serum titers of 1:8 (3 log 2) or lower were considered negative for antibodies against NDV. The GMT of the HI antibody titres was calculated for each batch from each of the farm.

RESULTS

Seven poultry enterprises agreed to participate in the study. The poultry farms were all from Sitiawan, Perak as this area has one of the largest concentrations of poultry farms. As observed, there were at least six vaccination types or protocols practiced by the seven poultry owners. Each of the protocols resulted in different outcomes of the vaccination in terms of the humoral HI antibodies elicited and the protection afforded. Referring to Table 1, 2 and 3, the GMT for the HI humoral antibodies for five of the farms were below standard, ranging from as low as 1.83 to 6.28 and this can be associated with its low level of protection afforded i.e as low as 38-76 %. The lowest percentage of protection afforded were 38% and 44% in chickens vaccinated with killed vaccines at 3 days old. The average antibody titre of the vaccinated flock may be satisfactory, however, there are still many birds that have poor antibody responses within the group. Three farms (Farms 4, 5 and 7) performed very well, where broilers were vaccinated with a killed followed by a live vaccine or by only the live vaccine i.e using La Sota strain.

Table 1: The types of vaccination practiced by the farmers, the age and HI-GMT at pre-challenged and the protection afforded after challenged with vNDV at $10^{4.3}$ EID$_{50}$ per bird, for broilers from each farm.

<table>
<thead>
<tr>
<th>Farm</th>
<th>Commercial ND vaccine and its combination</th>
<th>Types of ND vaccine virus</th>
<th>Age at vaccination</th>
<th>Vaccination Route</th>
<th>HI-GMT titre at (pre-challenged age)</th>
<th>Percentage protection against challenge with vNDV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ND + IB</td>
<td>Live</td>
<td>6 days old</td>
<td>Drinking water</td>
<td>2.9 (37 days)</td>
<td>76%</td>
</tr>
<tr>
<td>2</td>
<td>ND + IB</td>
<td>Killed</td>
<td>5 days old</td>
<td>Subcutaneous</td>
<td>1.83 (35 days)</td>
<td>38%</td>
</tr>
<tr>
<td>3</td>
<td>ND</td>
<td>Live (VG/GA strain)</td>
<td>1 day old at hatchery</td>
<td>Spray</td>
<td>4.70 (34 days)</td>
<td>62%</td>
</tr>
<tr>
<td>4</td>
<td>ND</td>
<td>Live (La Sota)</td>
<td>5 days old</td>
<td>Drinking water</td>
<td>115.36 (34 days)</td>
<td>100%</td>
</tr>
<tr>
<td>5</td>
<td>ND</td>
<td>Killed</td>
<td>3 days old</td>
<td>Subcutaneous</td>
<td>6.28 (27 days)</td>
<td>85%</td>
</tr>
<tr>
<td></td>
<td>ND</td>
<td>Live (La Sota)</td>
<td>7 days old</td>
<td>Drinking water</td>
<td>2.71 (30 days)</td>
<td>44%</td>
</tr>
<tr>
<td>6</td>
<td>ND + IB</td>
<td>Killed</td>
<td>3 days old</td>
<td>Subcutaneous</td>
<td>78.80 (34 days)</td>
<td>95%</td>
</tr>
<tr>
<td>7</td>
<td>ND</td>
<td>Live (ND F)</td>
<td>3 days old</td>
<td>Intranasal/ocular</td>
<td>95%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ND</td>
<td>Live (La Sota)</td>
<td>10 days old</td>
<td>Drinking water</td>
<td>95%</td>
<td></td>
</tr>
</tbody>
</table>
Table 2: The ND-HI titres at post vaccination i.e. before challenged and the outcomes after challenged depicted as dead (D) and HI titres for farm 1 – 7

<table>
<thead>
<tr>
<th>Chicken</th>
<th>HI GMT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.9</td>
</tr>
<tr>
<td>2</td>
<td>1.83</td>
</tr>
<tr>
<td>3</td>
<td>4.70</td>
</tr>
<tr>
<td>4</td>
<td>115.36</td>
</tr>
<tr>
<td>5</td>
<td>6.28</td>
</tr>
<tr>
<td>6</td>
<td>2.71</td>
</tr>
<tr>
<td>7</td>
<td>78.80</td>
</tr>
</tbody>
</table>

*%a/b (%) = no. survived (with HI titre)/Total no. of broiler tested (% protection)

<table>
<thead>
<tr>
<th>Farm 1 *13/17 (76%)</th>
<th>Farm 2 6/16 (38%)</th>
<th>Farm 3 8/13 (62%)</th>
<th>Farm 4 20/20 (100%)</th>
<th>Farm 5 *17/20 (85%)</th>
<th>Farm 6 7/16 (44%)</th>
<th>Farm 7 19/20 (95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before After</td>
<td>Before After</td>
<td>Before After</td>
<td>Before After</td>
<td>Before After</td>
<td>Before After</td>
<td>Before After</td>
</tr>
<tr>
<td>1&lt;2 D 8 256</td>
<td>&lt;2 D 256</td>
<td>32 64</td>
<td>4 1024</td>
<td>&lt;2 D 256</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 8 128 &lt;2 D 256</td>
<td>256</td>
<td>64</td>
<td>8 128</td>
<td>8 128</td>
<td>&lt;2 D 256</td>
<td></td>
</tr>
<tr>
<td>3&lt;2 1024 16</td>
<td>&lt;2 D 128 32</td>
<td>256</td>
<td>512</td>
<td>&lt;2 D 8 128</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4&lt;2 2048 128</td>
<td>&lt;2 D 128 32</td>
<td>256</td>
<td>512</td>
<td>&lt;2 D 8 128</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5&lt;2 8 32&lt;2 D 256</td>
<td>256</td>
<td>512</td>
<td>8 1024</td>
<td>4 D</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6&lt;2 1024 4</td>
<td>2048 4</td>
<td>1024</td>
<td>256</td>
<td>512</td>
<td>&lt;2 2048 32</td>
<td></td>
</tr>
<tr>
<td>7&lt;2 D &lt;2 &lt;2 2048</td>
<td>16</td>
<td>512</td>
<td>16 1024</td>
<td>16 128</td>
<td>256</td>
<td></td>
</tr>
<tr>
<td>8&lt;2 1024 &lt;2 D &lt;2 D 256</td>
<td>256</td>
<td>512</td>
<td>32 1024</td>
<td>16 D 256</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9 4 512 32</td>
<td>32 4</td>
<td>1024</td>
<td>64</td>
<td>32</td>
<td>&lt;2 D 8 32</td>
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<tr>
<td>10 64 128 &lt;2 D 32</td>
<td>64</td>
<td>32</td>
<td>64 128</td>
<td>&lt;2 D 32</td>
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<td></td>
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<tr>
<td>11 16 D &lt;2 512</td>
<td>&lt;2 D 8 16</td>
<td>16</td>
<td>512</td>
<td>&lt;2 D 64</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 16 128 &lt;2 D 32</td>
<td>64</td>
<td>128</td>
<td>128</td>
<td>128</td>
<td>&lt;2 512 128</td>
<td></td>
</tr>
<tr>
<td>13&lt;2 1024 &lt;2 D &lt;2 512</td>
<td>64</td>
<td>64</td>
<td>64 1024</td>
<td>32 D 32</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14&lt;2 D &lt;2 D 256</td>
<td>1024 16</td>
<td>2048</td>
<td>&lt;2 1024</td>
<td>64 256</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15 16 1024 &lt;2 D</td>
<td>32</td>
<td>128</td>
<td>4</td>
<td>1024</td>
<td>32 D 64</td>
<td></td>
</tr>
<tr>
<td>16&lt;2 32 &lt;2 D</td>
<td>16</td>
<td>256</td>
<td>32</td>
<td>1024</td>
<td>&lt;2 512 128</td>
<td></td>
</tr>
<tr>
<td>17&lt;2 2048</td>
<td>32</td>
<td>128</td>
<td>4</td>
<td>128</td>
<td>32 1024</td>
<td></td>
</tr>
<tr>
<td>18 128 256 &lt;2 D</td>
<td>256</td>
<td>128</td>
<td>16</td>
<td>512</td>
<td>128 512</td>
<td></td>
</tr>
<tr>
<td>19 64 128</td>
<td>16</td>
<td>512</td>
<td>128</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 128 64 &lt;2 32</td>
<td>128 32</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3: The no. and percentage of chickens with HI titre <2 for the seven farms that survived and died from the challenged with vNDV.

<table>
<thead>
<tr>
<th>Total no chickens with HI titre &lt;2</th>
<th>n = 44</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total no. of chickens that survived challenged</td>
<td>18</td>
<td>41% (survived)</td>
</tr>
<tr>
<td>Total no. of chickens that died after challenged</td>
<td>26</td>
<td>59% (died)</td>
</tr>
</tbody>
</table>
The HI - GMT of broilers at the pre-challenged titres was 115.36 and 78.8 and the protection afforded were 100% and 95% respectively. However, for broilers in Farm 5 with a GMT titre of 6.28, the vaccine seemed to provide reasonable protection of 85% against virulent ND virus challenged. This group of chicken was vaccinated with a killed followed by live La Sota vaccine strain via the subcutaneous and drinking water respectively. Compared to the vaccination regime using both live NDF and live La Sota strain via the intranasal/ocular and drinking water routes respectively, the vaccines seemed to invoke a much higher immune responses and afforded 95% protection to the vaccinated chickens. From the seven farms, 122 broilers were screened and challenged, and out of these 44 birds showed HI titres of < 2. Out of these 44 birds, 41% however, survived the challenge (Table 3).

DISCUSSION

For ND, the vaccines used for vaccination of large population of broiler chickens are usually the non-virulent live virus that is administered via spray or drinking water. Live virus vaccines provide acute antibody response, spreads systemically and invoke high cell mediated immune responses, thus providing greater and stronger protection to the chickens. However, the spray and drinking water methods of administering vaccines usually produce considerable variation in the individual immune responses, indicating potential variation in the levels of protection after vaccination (Senne et al., 2004).

Immune responses are determined via the HI test, the most widely used conventional serological method for detection of NDV antibodies as it is quite specific and gives reproducible results (Ricardo et al., 2000). Field results suggest that only birds with HI titers >16, usually after multiple vaccinations will survive a vNDV challenge and 66% of the flock with titres < 16 will not be protected and will succumbed to infection (Kaczynski and King, 2005). HI levels of 32 or higher have been typically shown to be protective against ND (Allan et al., 1978).

Vaccination against ND is easy as all NDV are in one serotype and any NDV strain can be used as a vaccine which will prevent clinical disease and death from ND. Lentogenic Newcastle disease (ND) vaccines indicated for young chicks usually do not evoke a strong and long lasting immunity. Although the average antibody titre of the vaccinated flock may be satisfactory, there are still many birds that have poor antibody responses within the flock. This is actually portrayed in the GMT value of the HI antibodies of the broilers of the seven farms, where at 27-37 days post-vaccination, The HI antibodies showing protective titres of ≥ 32 is only 4% (5/122). This is in agreement with many research that showed that lentogenic ND vaccinated flocks often do not achieve the required solid and uniform immune status following vaccination because a significant proportion of the population still remain susceptible. In the face of an outbreak, these flocks will experience some mortalities and a carrier status could be established in many of the infected birds (Senne et al., 2004). In Malaysia, challenged with field virulent ND virus could occur in chicks as young as 3 to 4 weeks of age and in such cases, disease control by vaccination would be difficult because the lentogenic vaccines which are safe for young chicks are unable to provide a full and solid protection against field virulent viruses. These seven farms can be considered fortunate as they might not have been hit by field virulent NDV, at the time of the study. It was observed that in two of the farms (Farm4 and 7), administration of live NDV vaccines induced very good antibody responses resulting in significant protection of the vaccinated birds. For Farm 5, concurrent vaccination with killed oil emulsion and live NDV vaccine also afforded 85% protection.

In this study, challenged was done through a milder normal route of infection i.e via intranasal, so as to simulate natural field infection. In the vaccination of broilers, taking into consideration the short life span of 45-50 days of broiler chickens, quick and high antibody responses is most desirable to protect them against ND. Inactivated or killed vaccines are often administered to layers and breeders as they provide long lasting high antibody titers that can be passed also to the offspring (Al-Garib et al., 2003). As killed vaccines are usually adjuvanted, they are given early in the life of the broiler chickens, due to withdrawal times problem between vaccination and slaughter. Furthermore, inactivated vaccines administered by the SQ route require individual administration, therefore, increasing also the cost of labour. Inactivated vaccines have been believed not to be able to induce a mucosal immunity, however, (Senne et al 2004) in a recent study, has demonstrated that both live and inactivated vaccines induced antibodies other than IgA not only in the serum but also in the tracheal and intestines (Chimeno Zoth et al., 2008). It was observed that for all the vaccination programmes of five of the farms, there were substantial numbers of chickens with antibodies < 2 at post-vaccination or prechallenged titres, where 59% died after challenge. The other 41% survived the challenged with some, also demonstrating a four-fold or more seroconversion. This protective phenomenon could be contributed by CMI mediated T and B lymphocytes which had been implicated in the development of protection in chickens vaccinated against NDV (Cannon and Russell, 1986; Sharma 1999; Reynolds and Maraga, 2000). One of the reasons why the 59% has HI antibody titles < 2 is that they might have missed the vaccine or have not received the full dosage of the vaccine.

This is a preliminary study on only seven farms and we hoped that it will provide insights into new research or investigations on how to help farmers know that the vaccines that they are using, the vaccination programmes and the vaccination procedures implemented will protect their chickens until marketing age. Knowledge on the one-time HI antibody level at slaughter will help farmers in reviewing their whole complete vaccination programmes and vaccines used, so farmers can formulate future strategies.
in vaccination to control ND. ‘Good’ or quality vaccines when administered correctly to healthy birds will help prevent death and disease. In cases of vaccine failure or poor vaccine take, farmers will need to reflect, identify reasons and review procedures pertaining to some of the important factors such as, storage, handling, dilution, vaccination routes and regimes and the types or strains of virus vaccines.

ACKNOWLEDGEMENTS

This study was conducted in the year 2005. The authors would like to thank Mr. Tan Siong Oh, Mr. Lee Ing Seng, Mr. Jailan Syait, Mr. Tan Swee Chai, Mr. Hoo Seow Chuan, Mr Cheong Pong Wow and Mr. Mohd Sham of the seven poultry farms in Sitiawan Perak for their participation and co-operation.

REFERENCES

DETECTION OF CAMPYLOBACTER AND SALMONELLA IN OSTRICH

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SUMMARY

Three ostrich show farms were visited to detect the presence of Campylobacter and Salmonella in the birds. Cloacal and skin swabs were taken from 31 ostriches which were clinically healthy. Campylobacter was isolated from one (1.6%) cloacal swab and Salmonella from two (3.2%) samples, a skin and a cloacal swabs; all were from different birds. Although the detection of the organisms was low, it could pose public health risk partly because of contact with the birds and the organisms in the faeces may contaminate the environment and it could be that they may be present in other birds but were not shed at the time of sampling.

Keywords: Salmonella, Campylobacter, ostriches

INTRODUCTION

Campylobacter and Salmonella are among the most important zoonotic pathogens causing acute gastroenteritis worldwide, particularly in industrialized countries (Cuomo et al, 2007). According to literature, Campylobacter species, primarily Campylobacter jejuni and C. coli, are reported to be commensals in many mammals and avian species which included chickens, ducks, turkeys, geese, ostriches and wild birds. Also, many animals in particular reptiles and birds are reported asymptomatic carriers of non-typhoidal Salmonella. These two organisms have been isolated in many outbreaks of campylobacteriosis and salmonellosis in humans with poultry meat and poultry products commonly implicated as the sources of the organisms.

Many studies have been carried out on the occurrence of Campylobacter and Salmonella in livestock and their products, pet and zoo animals and in wild birds; however, very little is known on the occurrence of the organisms in ostrich. Ostriches have been farmed since the middle of the nineteenth century, firstly in South Africa and subsequently in other countries for the principal purpose, until recently, for their feathers for use in the fashion industry and for industrial and household cleaning equipments (Gill et al., 2007). As the industry expands, more products have been produced from ostriches which include the hide (leather goods), variety meats (liver and heart), fresh meat (steaks and roasts), processed meats (sausage and ham type products) and health care products (Harris et al., 1993) These zoonotic organisms in the ostriches can cause diseases in humans when they come in contact with live or dead birds or upon consuming meat from infected ostriches, although for the latter the risk is minimal (Huchzermeyer, 1997).

Leisure activities in the show farms may expose the zoonotic organisms to visitors, especially if they consume food without proper washing of the hands after touching the animals or upon consumption of undercooked or contaminated cooked meat.

In Malaysia, ostrich farming is at infant stage as compared to Asia-Pacific region (China, Australia, Indonesia, Tasmania) (Leong, 2010). Thus, the objective of the study was to detect the presence of Campylobacter spp. and Salmonella spp. in ostrich.

MATERIALS AND METHODS

Collection of samples

The samples were collected from three ostrich show farms, with eight ostriches from Farm A, 11 ostriches from Farm B and 12 ostriches from farm C, a total of 31 birds. The ostriches were restrained by experienced staff. The eyes and head of each ostrich were covered by a hood so as to block the vision; this would calm the bird and facilitate handling.

Four sterile swabs were used to collect samples which consisted of two cloaca and two skin swabs from each bird. One set of swabs was placed into individual Cary Blair transport medium (Oxoid) for the isolation of Campylobacter spp. and the other set was placed into individual Buffered Peptone Water (Oxoid) for the isolation of Salmonella spp. Then, the swabs were transported in ice-packed cool box to the Veterinary Public Health Laboratory, Faculty of Veterinary Medicine, Universiti Putra Malaysia, and were cultured within two to three hours.

Culture for isolation and identification

Campylobacter spp.

Each swab in the Cary Blair transport medium was streaked directly onto Campylobacter blood-free selective agar base (Modified CCDA- Preston, Oxoid CM0739) incorporated with CCDA selective supplement (Oxoid

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The plates were incubated at 42°C for 48 hours under microaerophilic condition (5% oxygen, 10% carbon dioxide and 85% nitrogen) generated using a gas generating pack (BD Campy Pak) in an anaerobic jar. Each typical colony of *Campylobacter* was subjected to Gram-staining for cellular morphology and 'hanging drop' technique for motility. The presumptive colonies of *Campylobacter* spp. were subcultured on Columbia Blood agar (Oxoid) with 5% defibrinated horse blood added, and incubated at 42° C for 48 hours to obtain pure culture for biochemical tests. The tests included catalase, oxidase, urease, indoxyl acetate hydrolysis and hippurate hydrolysis tests. The isolated *Campylobacter* spp. was then kept in Brucella broth (Oxoid) with 10% glycerol added and stored at -20° C.

*Salmonella* spp.

Each swab in Buffered Peptone Water (Oxoid) was pre-enriched by incubating at 37° C for 24 hours under aerobic condition. Then, 1 ml of each pre-enriched broth culture was transferred into 10 ml of Rappaport Vassildalis broth (RV) (Oxoid) enrichment broth and was incubated at 42° C for 24 hours. One loopful of each enriched broth culture was streaked onto Xylose-Lysine-Tergitol-4® (XLT4) agar (Merck) and another loopful was streaked onto *Salmonella* Chromogenic agar incorporated with *Salmonella* selective supplement (Oxoid) and were incubated at 37° C for 24 to 48 hours under aerobic condition. The presumptive *Salmonella* colonies were gram-stained and subcultured. Biochemical tests which included Triple Sugar Iron (TSI) and Lysine Iron Agar (LIA) and urease tests were performed. The colonies that showed typical reactions of *Salmonella* spp. were then subjected to slide agglutination test (SAT) using *Salmonella* polyvalent ‘O’ antisera A-S.

**RESULTS AND DISCUSSION**

The *Campylobacter* colonies appeared as irregular shape, moist, slightly raised, grey, mucoid, discrete colonies and often as flat spreading colonies; the organism is Gram-negative, seen as slender, curved, S- or gull-wing shaped with corkscrew or darting movement. Only one sample (1.6%) from a cloacal swab of an ostrich in Farm A was positive for *Campylobacter*. Typical *Salmonella* spp. colonies appeared as red to yellow colonies with black centers on XLT4 agar and as magenta to purple colonies on the Chromogenic agar. They were seen as Gram-negative rods on Gram-staining. Two samples (3.2%) were positive, which were from a skin swab in Farm B and a cloacal swab in Farm C.

*Campylobacter* colonizes the intestinal mucus found on the outer layer and in the crypts as a commensal organism (Beery et al., 1988) and the principal site of *Salmonella* colonization is the caecum (Xu et al., 1988). Hence, both bacteria are most likely to be shed in the faeces. These bacteria may contaminate farm surfaces and environment which in turn may contaminate the feathers and skin of the birds which can contribute to the contamination of the carcasses at processing. Humans may come in contact with the organisms such as upon handling and touching the animals. *Salmonella* spp. can cause mortality in ostrich chicks (de Freitas Neto et al., 2009). The first isolation of *Salmonella pottsdam* in Malaysia from ostrich chicks with yolk sac infection was reported by Jasni et al. in 1998 (Zurina, 2005).

The low detection of *Campylobacter* in the study may reflect the true status in ostrich in Malaysia. However, it may also be due to failure to isolate the organisms as they were not shed at the time of sampling. Being fastidious organisms that require microaerophilic condition and capnophilic environment to survive (Mahon et al., 2007), *Campylobacter* spp. may resort to ‘viable but non-culturable form’ if they were in adverse or stress condition. Another possibility is that the number of organisms on the swabs was too small, thus enrichment broth should be have been used prior to plating (Bartelt, 2000). Enrichment stage is also important to revive the injured bacteria cells. Incubation temperature may also affect the number of *Campylobacter* spp. isolated. Incubation temperature used in this study was 42°C which is usually used to incubate enteric *Campylobacter* which are thermophilic such as *C. jejuni* subspecies jejuni. However, this is not suitable for non-thermophilic *Campylobacter* such as *C. fetus* subspecies fetus which are usually incubated at 37°C but this species is more commonly found in ruminants. According to Bartelt (2000), the growth of non-thermophilic campylobacters is inhibited at 42°C and thus no growth on the agar was observed. Some *Campylobacter* were susceptible to cefoperazone which was present in the CCDA agar, such as *Campylobacter upsaliensis* according to Aspinall et al., (1993), however this species is seldom isolated from avian species. Very few studies were available regarding the prevalence of *Campylobacter* in ostrich. Cuomo et al (2007) reported 40% of the clinically healthy ostriches in four farms in Italy were infected with *Campylobacter*.

There were a number of studies on *Salmonella* in ostrich that were accessible. The low presence of *Salmonella* in this study was similarly shown by Harris et al. (1993), who isolated *Salmonella* from 5.6 % of the surface and 20% of the faecal or caecal swabs of the ostriches in slaughterhouses in Texas, US. On the other hand, Vanhooser and Welsh (1995) isolated a higher number of *Salmonella* spp in the intestines and caeca of ostriches at 18.5% (46/248); *Salmonella* were isolated from clinically infected ostriches and those with subclinical infection and these birds had fence-to-fence contact with other animal species. In this study, the ostriches were clinically healthy and had almost no contact with other animals as they were kept in separate areas in the farm. de Freitas Neto et al. (2009) did not detect *Salmonella* spp. in
any droppings, eggs, caecal contents, swabs of carcases, spleens and livers from ostriches in production chain in Brazil; nevertheless, Salmonella Javiana and Salmonella enteric subsp. enterica 4, 12: i- were isolated from 2 out of 30 feed samples. Feed may be an important source of Salmonella spp. in ratite production which may be contaminated during processing or storage, and, as a result, it could play an important role in the introduction or maintenance of Salmonella spp. in ratite farms (Gopo & Banda, 1997; Higgins et al., 1997). There were studies that found high occurrences of Salmonella in ostriches. The study by Gaedirelwe and Sebuny (2008) in Botswana found the highest isolation of Salmonella in ostrich at 51.6% (16/31) was from the cloacae, while 13%, 16% and 29% from the livers, small intestines and large intestines respectively. Gopo and Banda (1997) found Salmonella on 51% of the ostrich’s surfaces (feathers) on arrival at the abattoir and on 8.3% of the skins. According to Friedman et al. (1998), the direct or indirect contact with animals colonized with Salmonella is another source of infection in humans, including contact during visits to petting zoos and farms. A report on an outbreak of salmonellosis among children was caused by an exposure to a reptile exhibit at a zoo; the visitors had touched the barriers which had been touched by the Salmonella-infected reptile and that they had placed their contaminated hands in their mouth or cross contaminated the food eaten (Friedman et al., 1998). As in the case of Campylobacter, the low presence of Salmonella spp. in the study may indicate the true status in the farm or the organisms were not shed at the time of sampling.

The comparison in the occurrences of pathogens among studies is not truly possible even if similar samplings and methods of isolation and identification are used; this is because the exposures to the organisms and risk factors involved may differ among animals, farm environment and locations. It is well recognised that molecular methods such as PCR assay are said to be better in detection of pathogens compared to conventional microbiological method even though very selective and chromogenic agar are used, however at times the results obtained are often comparable. According to Gopo and Banda (1997), molecular methods are more reliable and specific as they do not show any false negatives, but they can be expensive as special equipment and reagents and trained personnel are required to carry out the work.

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PORCINE CONGENITAL SPLAYLEG: A REVIEW

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SUMMARY

Porcine congenital splayleg (PCS) is a clinical condition of newborn piglets, characterised by muscle weakness, resulting in the inability to properly stand and walk, with affected limbs extended sideways or forwards. It is arguably the most important congenital defect of commercial piglets and causes significant economic loss to pig farmers. The aetiology and pathogenetic mechanisms for PCS are still not well understood. Various management, nutrition and genetic factors have been found associated with PCS problems, but the actual cause remain obscure. Proper management and good farm practise are essential to control this multi-factorials PCS problem.

Keywords: congenital splayleg, porcine, piglet, skeletal muscle

Introduction

Preweaning mortality in piglets constitutes a major loss to the pig industry. Congenital abnormalities account for a small but significant proportion of preweaning losses (Partlow et al., 1993). They have been reported more frequently in pigs than in any other domestic animal species (Priester et al., 1970). A number of abnormalities may be seen in newborn piglets or shortly after birth. Briefly, congenital conditions can be categorised as: (1) spontaneous developmental abnormalities, such as cystic lymph nodes, (2) heritable abnormalities such as congenital meningoencephalocele, atresia ani, arthrogryposis, porcine congenital splayleg, (3) infectious agents such as congenital tremor type AI (myocloniacongenita), (4) nutritional deficiency or poisoning such as mulberry heart disease and (5) unknown causes such as bleeding navel syndrome (Taylor, 2013).

Porcine congenital splayleg (PCS), also known as straddlers or myofibrillar hypoplasia, is a clinical disease of newborn piglets. It is arguably the most important congenital defect of newborn piglets and causes significant economic impact to the industry. PCS is the inability of newborn pigs to stand and walk properly, often with their limbs extending forwards or sideways as a result of muscular weakness (Thurley et al., 1967).

In 1967, the clinical term was first reported by Thurley et al. (1967). Since then, reports from different countries regarding congenital splayleg were published (Dobson, 1968; Cunha, 1968; Olson and Prange, 1968; Bollwahn and Pfeiffer, 1969; Lax, 1971; Svendsen and Andereasson, 1980). Reports on the prevalence vary considerably. In the United Kingdom, Ward and Bradley (1980) estimated that about 0.4% piglets were affected by splayleg, which caused an annual loss of £300,000 to the pig industry at that time. In another two studies by Dobson (1968, 1971), the overall average prevalence rates were reported at 11% and 13% respectively. A further study in Ontario indicated that PCS was the most common congenital defect with 0.87% piglets affected (Partlow et al., 1993).

Clinical signs

PCS can be found at or a few hours after birth. At birth, 2 to 3 piglets may be affected in each litter. It invariably affects the hind limbs, but occasionally affects the forelimbs. Most of the affected piglets are unable to move around or stand, although some splayleg pigs may able to move around with difficulty. The affected limbs are abducted, splayed forward or in sideways position. Often, an affected piglet is found seated on its hindquarters (Figure 1).

Figure 1.Porcine congenital splayleg. A 2-day-old piglet with PCS showing forward extension and abduction of hindlimbs as a consequence of muscle weakness.

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An affected piglet is often separate from its healthy litter mates. Its immobility means that it cannot gain sufficient nutrients from the sow, resulting in starvation and hypothermia. Affected neonatal pigs become emaciated, weak and dirty. Abrasions and ulceration develop on the body due to long periods of lying on the floor. Splayleg pigs are more predisposed to arthritis, polyarthritis, pododermatitis and osteomyelitis of the digits due to secondary bacterial infections. The mortality rate of splayleg piglets can reach around 50%. The cause of death is either starvation or crushing by the sow. However, if supportive treatment and extra care can be provided, the affected piglet can recover after one week.

Prevalence and incidence

The condition is particularly prevalent in Landrace and Large White breeds, which are heavily muscled (Dobson, 1968; Tomko, 1993; Vogt et al., 1984), although it is known to occur in virtually all commercial lines. Both male and female piglets are susceptible to PCS (Tomko, 1993; Thurley et al., 1967), however, some studies indicate that male offspring are more susceptible. In one of the studies, male progeny are 1.74 times more likely than females to succumb to PCS (Vogt et al., 1984). Another study showed that about twice as many males were affected as females (Van Der Heyde et al., 1989). Presently, it is not clear whether PCS is related to birth weight or litter size. One study found that PCS occurs more frequently in large litters than in smaller ones (Van Der Heyde et al., 1989). However, another study suggested that the occurrence of splayleg was significantly higher in small litters (Tomko, 1993).

Pathogenesis of PCS

The aetiology and pathogenesis of PCS are not known. It is considered to be a multifactorial condition. The factors that are involved are thought to include genetic and environmental factors like sow management, administration of various drugs and mycotoxins.

Treatment of pregnant sows with glucocorticoids can induce a myopathy in the newborn which mimics PCS (Jirmanova, 1983). However, there were histological differences in muscle from glucocorticoid induced and naturally occurring PCS (Ducatelle et al., 1986). Dexamethasone is a synthetic glucocorticoid used primarily as an anti-inflammatory agent in various conditions, including allergic states. More recently, it was suggested that affected muscles have a reduced number of myofibrils and an increased accumulation of glycogen when compared with the muscles of normal piglets. The investigation had concentrated on the activity of glucose-6-phosphatase (G-6-Pase), a liver enzyme that breaks down glycogen reserves (Antalikova et al., 1996). Another study suggested that the slippery floor in farrowing crates could predispose newborns to PCS problems (Dobson, 1971). However, this environmental factor is not likely to be a causal factor of splayleg (Van Der Heyde et al., 1989). Choline is a vitamin like compound which is essential for acetylcholine synthesis. Supplementation with 2.2 to 3.0g of choline in the diet of pregnant sow until parturition was reported to reduce the incidence of PCS (Cunha, 1968). However, a subsequent study could not reproduce this finding (Dobson, 1971).

Consumption of fusarium F-2 toxin (zearalenone) contaminated grain by sows in late pregnancy, could lead to a higher incidence of PCS, a condition that can be experimentally reproduced by the administration of purified F-2 toxin to pregnant sows (Miller et al., 1973). Increased stillbirths and neonatal mortality were also recorded. However, F-2 induced PCS piglets did not show the typical histopathological lesions described by Thurley et al. (1967).

Misuse of certain drugs in pregnant sows may lead to signs of PCS in newborn piglets. Administration of 3.6mg/kg/day of pyrimethamine, an anthelmintic, to pregnant Goettingen minipigs raised the incidence of PCS to 74% of the newborn, while the control group had only an incidence of 5.6% (Ohnishi et al., 1989). Induction by prostaglandin before the 113th day of pregnancy could also lead to higher incidence of congenital myofibrillar hypoplasia (Bolcskei et al., 1996).

Genetic factors play an important role in PCS as well. Porcine CDKN3 gene (cycline-dependent kinase inhibitor3) which involved in cell cycling was strongly displayed in splayleg muscle (Maak et al., 2003). Atrophy marker gene FBXO32 (atrogin, MAFbx) was found to be highly expressed in PCS samples (Ooi et al., 2006). Recently, another four genes which are sequesosome1 (SQSTM1), structure specific recognition protein 1 (SSRP1), v-maf musculoapneurotic fibrosarcoma oncogene homolog (MAF) and DNA-damage-inducible transcript 1 (DDIT4) are indicated might involve in PCS pathway as different expression levels were detected comparing genome wide gene expression of three hind leg muscles (muscle adductores, muscle gracilis and musclesartorius) between splayleg piglets and their healthy litter mates (Maak et al., 2009).

Histopathology of PCS

A variety of lesions have been described as the underlying pathological changes in congenital splayleg. The most consistent change is the presence of so-called myofibrillar hypoplasia (MFH), interpreted as an immaturity of the muscle (Ducatelle et al., 1986; Thurley et al., 1967). Myofibrillar hypoplasia ranges from a slight reduction of myofibrillar content to severe myofibrillar deficiency, vacuolisation, focal degeneration and necrosis. However, myofibrillar hypoplasia can also be found in clinically normal piglets. The term congenital myofibrillar hypoplasia may not therefore be the diagnostic description of PCS (Ducatelle et al., 1986).
As pointed out earlier, the morphological findings of dexamethasone treatment suggest that PCS might represent a congenital form of glucocorticoid myopathy (Ducatelle et al., 1986). Another study on muscle ultrastructure of PCS piglet, also showed reduced numbers of myofibrils and an increase in glycogen accumulation in comparison with muscle of normal piglets (Antalikova et al., 1996). Other studies found differences between PCS piglets and piglets with experimentally-induced glucocorticoid myopathy. Naturally occurring PCS had glycogen-filled extramyofibrillar space (EMS), whereas dexamethasone splayleg had only limited glycogen in the EMS (Ducatelle et al., 1986).

There were no significant qualitative differences between normal pigs and splayleg pigs aged from birth to 1 week, based on light microscopic and ultrastructural examinations (Bradley et al., 1980; Ward and Bradley, 1980). The progressive clinical improvement in splayleg during the first week of life was found to be accompanied by an increase in muscle cell size, a reduction in the number of nuclei, a reduction in the severity of MFH, a reduction in the size of the extra-myofibrillar space and an increase in intracellular lipid. However similar changes were also found in normal pigs. The results indicated that light microscopy or ultrastructural morphology were not useful to diagnose splayleg due to the failure to detect any significant differences (Ward and Bradley, 1980). However, both studies took the form of visual subjective assessments. Quantitative measurements were not performed. A congenital, impaired functionality of skeletal hind limb muscles due to immaturity and/or atrophic properties is likely to be the major patho-morphological features in PCS (Maak et al., 2009).

Control and treatment of PCS

Given the uncertain aetiology and pathogenesis of PCS, it is difficult to reduce its incidence. From the husbandry viewpoint, a dry and non-slippery floor should be provided for farrowing. In addition, neonatal piglets should be protected from injury by the sow, and provided with adequate opportunities to suckle. These could reduce the incidence or the severity of PCS. Selection of breeding stock may help to control the disease. Good farm breeding records can help to identify individuals that are predisposed to the production of affected offspring. In addition, affected piglets that recover should not be used for breeding. With supportive care, adequate warmth and nutrition, affected piglets can recover from the condition. Some farmers suggested by providing sufficient of Vitamin C, the problem will subside. However, nursing is labour consuming and may not be economical as a routine in herd health management. The common treatment method used is tying together the affected limbs, with a loose “figure of 8” just above the hock joint. It appears to help the piglet to recover and gain the ability to move around. Adhesive tape can be used to tie affected legs but care must be exercised to avoid occlusion of blood flow.

REFERENCES


HELCOBACTER INFECTION IN PET AND FOOD ANIMALS: OCCURRENCE AND ZOONOTIC POTENTIAL

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Summary

The presence of Helicobacter spp. in the stomach of pet and other animal species has been reported worldwide but the documentation of occurrence of the organism in these animals in Malaysia is lacking. This paper discussed the occurrence of helicobacters in cats and dogs (pet animals) as well as in chickens and pigs (food animals) and the zoonotic potential of these organisms in animals that may infections cause in humans. The occurrence of helicobacters in animals in Malaysia from limited available studies is presented.

Keywords: helicobacters, cats, dogs, chickens, birds, zoonotic implications

INTRODUCTION

Helicobacters are gastric spiral- or curved- shaped, gram negative microorganism which have been observed in animals and humans for more than a century. In animals, these microorganisms were first described by Rappin in 1881 in the stomachs of dogs and cats (Cattoli et al., 1996). It was only in 1982 when Helicobacter pylori was discovered in diseased gastric tissue of humans and that the subsequent recognition of its prevalence and clinical importance as a cause of gastric ulcers in man, that led to the studies of these organisms in animals with renewed and increased interest.

To date, there are at least 32 species of helicobacters, isolated mainly from the stomachs, intestines and also livers of various animals, including dogs, cats, ferrets, monkeys, pigs, sheep, rats, mice, hamsters, chicken, and birds as well as man (Haesebrouck et al., 2009; Fox, 2002; Fox, 1999; Jalava et al., 1998; Wesley, 1997) (Table 1). These species may be grouped as gastric helicobacter species causing enteric disorders and enterohepatic helicobacter species which are associated with hepatobiliary diseases (Solnick and Schaeur, 2001; Milosavljevic, 2001). In man, H. pylori is well known and most importantly in terms of its impact on human health; other helicobacters species that are associated with gastric and hepatic diseases are termed as non-H. pylori helicobacters (NHPHs) (Fox, 2002; Milosavljevic, 2001).

The paper discussed the occurrence of helicobacters in pet animals, particularly cats and dogs, avian species namely chickens and pigs and their potential public health significance. Cats and dogs are among the most kept pet animals and chickens and pigs are intensively reared to provide meat which are widely consumed. Although ruminants too are intensively reared for meat production, however information regarding the occurrence of helicobacters in these ruminants are very scarce.

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Prevalence of Helicobacter infections in cats and dogs

The diagnosis on the presence of helicobacters in mammalian animals is mostly through the use of invasive methods, involving the analysis of gastric biopsy tissues usually obtained during gastric endoscopy. These biopsies are subjected to urease production testing, microbiological culture, gram staining, histological examination with Haemotoxylin and Eosin (H&E) or Warthin-Starry silver staining and electron microscopy. There are difficulties in the isolation of helicobacters due to their fastidious nature and also considerable problems in accurately identifying Helicobacter spp. using conventional methods, therefore molecular techniques are preferred. These molecular techniques include polymerase chain reaction (PCR), amplified fragment length polymorphism (AFLP), restriction fragment length polymorphism (RFLP) and 16s rRNA gene sequencing. The non-invasive methods involve serology (the measurement of circulating antibodies (IgG)) and urea breath test; the latter test reported as extremely useful for the diagnosis of helicobacteriosis in humans, monkeys, ferrets and pigs and for assessing their response to treatment (Simpson and Burrows, 1997).

Helicobacter felis, a ‘spirillum’ originally isolated from the cat stomach is also shown to colonize dogs (Otto et al., 1994). To date, there are number of species found to colonize the gastric mucosa of cats and dogs as well as other animals (Table 1). On occasion, cats have been found to harbour H. pylori (Handt et al., 1994). Helicobacter bizzozeronii (also referred to as Gastroprilliumor Helicobacter heilmannii) and H. felis are collectively termed as gastric helicobacter-like organism or GHLOs. Depending on types of detection methods and locations, the presence of helicobacters in gastric biopsy tissues of cats and dogs ranged from 41% and up to 100%. Apart from gastric mucosae, a recent study by Tabrizi et al. (2010) found Helicobacter spp. in 93% of oral secretions of stray cats while Recordati et al. (2007) detected 71% of dogs
harbour Helicobacter spp. DNA in the oral cavity (dental plaque and/or saliva).

**Occurrence of Helicobacter in food animals**

With regard to helicobacters in food animals, reports were mainly available on their occurrence in poultry and pigs. Unlike the helicobacters in cats and dogs, helicobacters in poultry were isolated from intestinal or caecal contents, faeces, livers and carcasses. The samples were isolated using culture media, identified using extensive phenotypic testing and confirmed using molecular techniques as mentioned above. In a number of studies which isolated Campylobacter-like organism (CLOs) from poultry, upon further analyses such as using nucleic acid technique (nucleotide sequence of the 16S rRNA gene) and whole cell protein analysis (SDS-PAGE), identified them as Helicobacter pullorum (Stanley et al., 1994; Attabay et al., 1998). CLOs were also found in other animals, such as cats and pigs which were later identified as Helicobacter species (Patterson et al., 2003; Shen et al., 2001).

In avian species, which are commonly colonized with thermophilic campylobacters, a technique is needed for rapid detection and differentiation of these organisms from H. pullorum; the ability and efficacy of multiplex PCR (Neubauer and Hess, 2003; Miller et al., 2006) and PCR-RFLP analysis of the 16S rRNA gene (Marshall et al., 1999) has been reported. A number of studies isolated H. pullorum in chickens, such as Ceelen et al. (2006) in Belgium found 34% and 32% H. pullorum in caeca and colon respectively, 11% of jejenum and 5% of liver of broiler chickens, Miller et al. (2006) in Australia found 27% in broilers in one farm and Zanoni et al. (2007) in Italy reported all 60 chickens from 15 farms were positive for H. pullorum. The organisms were found in 76% of turkeys (Zanoni et al., 2011).

It is reported that H. suis is the main Helicobacter species colonizing the stomachs of pigs. Its prevalence in pigs at slaughter age is 60% or more (Haesebrouck et al., 2009). Park et al. (2004) examined the stomach samples of 10 pigs of 6-months of age after slaughter and found the 95% infection rate of Helicobacter spp. using PCR assay compared to 62.5% when using silver staining. Pigs may also be infected with H. heilmannii as high as 100% (Solnick and Schauer, 2001).

**Occurrence of Helicobacter in cats, dogs and chickens in Malaysia**

To date, there is very scanty published data on the prevalence of helicobacters in cats and dogs in the country. In a preliminary study by Ravindran (2002), 80% of the gastric biopsy samples from 30 cats were positive by rapid urease test; while 20% of the gram stained-direct smears of the samples were similar to those described by Lee et al. (1988) and Otto et al. (1994). On culture, only one sample (3.3%) showed growth with a thin film watery-like appearance which was positive for Helicobacter spp. In another study by Nur Zaliza (2004) on the gastric biopsy samples of 30 cats and dogs, it was reported that 66.0% were positive by rapid urease test, 16.6% were presumptively identified as helicobacters and on culture, 36.6% showed growth. Also in this study, upon using the PCR technique, 16.6% of the gastric mucosa samples were positive for Helicobacter species.

The occurrence of helicobacters in chickens was reported in a study by Soe Soe Wai et al. (2012); H. pullorum was isolated from 25% village chickens from five markets and 24.6% in broiler chickens in six farms using culture methods and confirmed by PCR assay.

**Public Health Significant**

It has been reported that domestic animals may serve as reservoirs for human Helicobacter infection. It was discovered that a small subset of human gastritis cases were not caused by H. pylori but by other gastric helicobacter-like organism (GHLOs) which were reported to be morphologically identical to those found in animals (Otto et al., 1994). Table 1 shows helicobacters that are zoonotic or has potential as they have been reported to occur in both human and animals (Haesebrouck et al., 2009; Fox, 2002; Solnick and Schauer, 2001; Fox, 1999). In recent years, there has been concern over pet animals, in particular cats and dogs, and pork and chicken meat, as the source of Helicobacter infection in man. Four modes of transmission have been proposed for H. pylori which are faecal-oral spread, oral-oral spread via salivary secretions, pet-to-human as well as human-to-pet transmission and ingestion of contaminated food and water (Wesley, 1997). In man, H. pylori is the major agent of chronic diffuse superficial gastritis, plays causative role in peptic ulcers and is considered a cofactor in the development of gastric cancers (Neiger et al., 1998).

Several suggestions have been made that among pet animals, cats are more likely a potential reservoir of H. pylori. Handt et al. (1994) cultured H. pylori from six young adult cats and reported the possibility that H. pylori infected may be a zoonotic disease, with transmission occurring from cats to humans. Fox et al. (1999) reported that H. pylori was cultured from salivary secretions in 6 of 12 (50%) cats and from gastric fluid samples in 11 of 12 (91%) cats and from faeces in 4 of 5 (80%) cats upon PCR technique and amplifying H. pylori 26kDa surface protein. The possibility that H. pylori may be transmitted to humans from cats may also be brought about the facts that cats are popular pets and there is a significant cat-human contact; also, cats vomit occasionally and continuously grooming.
which may result in oral-oral route of transmission to man (Handt et al., 1994). It also suggested that since ferret shed viable gastric _H. mustelae_, there is a possibility of the presence of _H. pylori_ in cats' faeces which man can be exposed to upon cleaning the litter box. A more likely suggestion is that _H. pylori_ in cats may well be a reverse zoonosis (anthropozoonosis), whereby humans transmit the organism to cats (Niegger, 2003). _Helicobacter heilmannii_ and _H. felis_ were reported to colonize a small percentage of humans with gastritis; since the environmental source of these organisms has not been recognized, it is suggested that pets were the source of transmission to man (Fox, 2002). _Helicobacter heilmannii_ is said to cause chronic active gastritis in man and animals, such as cats, dogs, pigs and primates, it is caused mild to moderate gastritis. 80-100% of cats, dogs and pigs were reported to be infected with _H. heilmannii_ (Dieterich et al., 1998). Dieterich et al. (1998) reported the isolation of _H. heilmannii_ in a man who had a 4-years history of recurrent dyspepsia and from his two cats; however, there is the possibility that the cats and their owner became infected from the same source.

It is interesting to note that the overall _H. pylori_ prevalence of 49.0% was recorded in man in Malaysia, with the prevalence among the races as follows: Malay 16.4%, Chinese 48.5% and Indians 61.8% (Goh, 1997). According to Goh (1997), the hypothesis of a racial cohort phenomenon is based on the presumption that the both Chinese and Indians, being immigrant races, may have ‘brought over’ the infection from their countries of origin where the prevalence of _H. pylori_ is known to be high; even in Singapore, the Malay population has the lowest prevalence compared to the other two races. Interestingly too, according to Chow et al. (1995), the Chinese immigrants living in Melbourne (Australia) also showed higher prevalence of _H. pylori_ infection which was associated with age, birthplace and the use of chopsticks suggesting an oral-oral spread.

_Helicobacter pullorum_ was reported to cause serious gastroenteritis with chronic diarrhoea in man (Fox et al., 2002). The isolation of the organism from diarrhoeic humans and their presence in chicken faeces and carcasses led to suggestion that consumption of chickens may cause _H. pullorum_ infection in man (Atabay et al., 1998; Fox et al., 2002). A Swiss study on 387 _Campylobacter_ spp. Isolated from human patients with gastroenteritis, found six (1.5%) identified as _H. pullorum_; the authors suggested the possibility of transmission of the organism from poultry to humans (Burnens et al., 1994). Since _H. pullorum_ resembles _Campylobacter_ colit, it could be overlooked as a cause of human gastroenteritis.

The investigation by Van den Bulck et al. (2005) into the presence of NHPHs in gastric samples of human found that _H. suis_ (37%) and _H. salomonis_ (21%) to be the most prevalent _Helicobacter_ species encountered compared to other _Helicobacter_ species and a study reported a high rate (78%) of _H. suis_ in German patients. Thus, according to Van den Bulck et al. (2005) pork consumption and direct contact with pigs may constitute a risk factor for human NHPH infection.

In summary, the large number of _Helicobacter_ species isolated from animals and that some of these species were also found in human indicate the zoonotic potential of these species of helicobacters. Among these animals, cats and pigs are important in human gastric NHPH infection but other sources need to be investigated. Moreover, the wide range of animal species found to harbour helicobacters not only in their gastrointestinal tracts but also in the oral cavities may increase the possibility of human exposure to these organisms and as such, precautionary measures need to be taken upon handling animals.

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INTRODUCTION
Veterinarian perform a thorough examination, arrived at a correct diagnosis and recommended an appropriate treatment but poor compliance (Barter et al., 1996) from the owners and/or pets to the advice given will potentially cause therapeutic failure. Long-term medication of tablets prescription to companion animals are particularly challenging especially in non-compliant pet (i.e. aggressive, fierce, fear biter). The successfulness of a long-term treatment is heavily dependent on owner compliance, their willingness and ability to administer the prescribed medication. Therefore, a suitable and user-friendly way of administrating drugs needs to be identified.

Veterinary compounding drugs
In general, tablets are allowed for administration of therapy without presence the veterinarians but owners’ or companion animals’ compliance can be a problem. Owners may fail to administer the tablet properly, their pets may not consume the entire tablet or only partial dose was administered especially in cats as this species at times can be difficult to medicate. To assist drug delivery and to encourage compliance, drugs are sometimes compounded by veterinarians, veterinary pharmacists or compounding pharmacists. To date, many studies and reviews of veterinary compound drugs have been published. They generally aim to; 1) to enhance consumer convenience and compliance; 2) to improve the pharmacokinetics of drugs and; 3) to assure target and consumer safety (Ahmed and Kasraian, 2002; Merton Boothe, 2006; Papich, 2005). Drugs have been compounded for veterinary medical use because many were not in an ideal form of formulation to be used in the species being treated (cats, exotic animals and pet birds). To date, there are only a few approved veterinary formulations in the market (Hardee and Baggot, 1998).

An extemporaneously prepared compounding drug alters the original drug dosage formulation for ease of administration. Normally, conventional tablets will be crushed, capsules reformulated and solution altered to make it more convenient and palatable oral dosage formulation. Palatability, ways of administration, methods of dispensing and frequency of administration are factors that must be considered carefully when formulating a compounded oral drug for companion animals. The combination of these factors and the oral drug formulations produced must have good end result with regards to drug stability, purity and potency comparable to the original formulation (Hardee and Baggot, 1998; Papich, 2005).

The need of alternative formulations of drugs in veterinary medicine as well as in medical care for humans, particularly for use in paediatric patients, has lead to a boost of studies being conducted. Researchers have look into physiology function of the gastrointestinal tract of companion animals with drug performance (Sutton, 2004), comparison between different formulations in terms of drugs and products (e.g. in different packaging) stability assessment (Garner et al., 1994), photosensitivities studies (Andrisano et al., 1999), enantioselective behaviour and stereospecific of drug studies (Landoni et al., 1997; Mehvar and Brooks, 2001). There are also published surveys and feedbacks on compliance with medication prescribed (Barter et al., 1996), palatability studies (Hames et al., 2008; Litster et al., 2007) and preference of formulation (Cohen et al., 2009). However, many have given great emphasized on the pharmacokinetics, pharmacodynamic and bioavailability of drugs (Arguedas et al., 2008; Beddies et al., 2008; Buck et al., 1989; Flammer et al., 2008; Jug et al., 2009), all with the ultimate goal of producing a safe and suitable compounded oral drug formulation.

Oral formulation
Oral dosage consist of a large proportion of drugs formulations. Commonly, oral dosages are prepared in the form of solution, emulsion, suspension, gel/paste, powder, capsules and tablets. The solution, emulsion and suspension are generally in the form of liquid administered orally with an aid of a syringe. Where else, the gel and paste are semisolid precise oral dosage application on the upper gum or palate commonly supplied in pre-loaded calibrated syringes. The rate of paste drugs absorption would be expected slower than from a liquid but faster than the solid dosage form. Capsule is an easily digested and tasteless unit dose containers which allow accurate amount of drugs to be contain within a capsule. Different oral drug formulations such as powders, granules, pellets, suspension, emulsion or oils measured could capsule but commonly intended for human usage and often contain an inappropriate dose for most animal species. Oral powder formulations are put on food during feeding time and must be palatable (Hardee and Baggot, 1998).

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However, conventional tablets are the most widely used oral drug administration in small animals. It has certain advantages over oral liquid dosage form. A tablet contains an equivalent dose of active drug in a compact form, easier to administer and usually presents the fewest problems with regard to stability. Bioavailability of a drug can vary widely among tablets because of the wide range of body weight, the total dose requirements of different species, the strength of the tablet (amount of the drug contained therein) largely determines its suitability for use in a particular species. Therefore, tablets are sometimes cut to avoid overdosing and this may lead to inaccuracy of medication if tablets were not divided properly (Hardee and Baggot, 1998).

On top of that, tablet administrations are very challenging in non-compliant pets i.e. cats as they are notorious for ejection of tablets within second of their administration. In this circumstances, formulations such as liquid and paste may be better alternative or adding powder and granules into the food may be more convenient in non-compliant cats (Hardee and Baggot, 1998). Therefore, the pharmacodynamic, pharmacokinetics and bioavailability of different formulations of the same drug should be conducted and correlated to owners’ and cats’ compliance. It is crucial to determine which formulation would be cats’ and owners’ preference, good compliance practiced and achieved a good therapeutic effect. This applied the same for other species of companion animals too.

A few veterinary pharmaceutical companies have commercially produced drugs in other formulation and many veterinarians have compounded drugs in improve their therapeutic effectiveness. Veterinarians have assumed commonly that compounded oral formulations perform as well as the original formulation. However, this assumption should be investigated. Improper prescription of ineffectively compounded oral drug formulation would waste pet owners’ time and potentially put at risk the health of the companion animal. Besides that, there is also a lack of studies on pet owners and companion animal compliance with regards to the usage of compounded oral formulation. More studies could provide useful feedback for pharmaceutical companies. This could lead to more compounded oral formulations of different drugs to be marketed and there could be a change in trends in oral drug formulation used by veterinarians.

**Drug compliance**

Drug compliance is generally described as the adherence of patients to their prescribed medication in human medicine (Besch, 1995; Cramer and Spilker, 1991; Haynes et al., 1979). Therefore, drug compliance in veterinary medicine can be defined as the extent to which owners adhere to instruction when giving prescribed drugs to their animals. To date, veterinary drug compliance in veterinary medicine has been reported to range from 44% to 55% and human medicine has a wider percentage in comparison at 5% to 96% (Berendsen and Knol, 2002; Cramer and Spilker, 1991). The range of compliance supports the speculation in human medicine that patients do not adhere strictly to instructions for the use of medication. Poor compliance with drug therapy is widespread in all aspects of human medicine due to many factors (Cramer and Spilker, 1991; Haynes et al., 1979; Mackner and Crandall, 2005). As animals are dependent upon their owner for administration of medication, there is every reason to assume that non-compliance is prevalent in veterinary medicine.

Therefore, alternative compounded drugs produced which have comparable or superior pharmacokinetic or pharmacodynamic to its original form should be investigated. The new alternative compounded formulation that may probably be not user friendly would defeat the purpose of effective therapeutic treatment and aid in compliance.

**How to assess levels of compliance in veterinary medicine**

Drug compliance has been measured and methods have been compared in several ways to assess levels of compliance directly (e.g. measurements of drugs in blood and urine excretion, measurements of biological or inert markers) or indirectly (e.g. therapeutic outcomes, clinical opinions, interviews, filling of prescriptions, pill counts, microelectronic monitors) (Besch, 1995; Cramer and Spilker, 1991; Haynes et al., 1979). There is no one valid, reliable or novel method to assess drug compliance levels in veterinary and human medicine (Andersen et al., 1995; Barter et al., 1996; Cramer et al., 1989; D'Souza et al., 1983; Paes et al., 1998; Partridge et al., 2001; Udelson et al., 2009). Most of the studies on drug compliance in veterinary medicine are based on short courses of medication in dogs (Barter et al., 1996; Bomzon, 1978; Grave and Tanem, 1999) and no one has looked at drug compliance of cats and pet owners.

Pill counts were the simplest and cheapest method adopted but results obtained could be an over- and underestimation of compliance level as pet owners could give more pills than required or dispose of them (Bomzon, 1978; Grave and Tanem, 1999). Electronic monitoring involves placing a microchip on the container lid which records the number of times the lid was opened in a day. This method is expensive and could arouse suspicions as the containers looks different from normal dispensing containers. Pet owners might remove more than one dose or no dose while opening the lid which would reflect over- or under dosing (Barter et al., 1996). Compliance in dosing intervals can be determined from the electronic monitoring which provides objective compliance measurements of daily dosing pattern and interval. This assessment is crucial as over-, under and erratic dosing intervals can diminish drug actions or cause adverse effects (Barter et al., 1996). Therapeutic outcomes and monitoring with drug assays
using analytical techniques can be used to measured levels of compliance but such assays are expensive and subjected to individual pharmacokinetic variability. Veterinarians’ assessment of client compliance (predictability) has been used but the validity of such a subjective and indirect measurement is questionable as veterinarians can overestimate the level of client compliance (Barter et al., 1996). Owner self reports, interviews and questionnaires are methods by which owners are ask directly or indirectly regarding their compliance. These are simple and inexpensive methods that can easily be conducted in a veterinary practice. These methods allow pet owners to express problems encountered during drug administration and their observations (Barter et al., 1996; Bomzon, 1978; Grave and Tanem, 1999; Litster et al., 2007).

Factors affecting compliance

There are other factors that affect compliance levels besides the role of pet owners as administrators. Ease of administration is an important consideration when a drug is formulated. Generally, cats and dogs are administered tablets by placing the tablet at the base of their tongues (far back) and gently “poke down” the medication. The pet’s mouth is quickly closed, the head returned back to the normal position and the throat is massaged or animal is distracted till medication is swallowed. Often, this is easier said than done particularly in cats as this species is more independent and less accustomed to being restrained. Also, there is the owners’ fear of being clawed or bitten (Thombre, 2004). Therefore, the compliance level tends to decrease when a particular formulation is not user-friendly. To assist therapeutic treatment, alternative formulations which are easy to administer such as oral administration (solutions, suspension, paste/gels, capsules, powder/ granules) (Hardee and Baggot, 1998) and transdermal application (ointment, cream, liquid) (MacGregor et al., 2008; Magnusson et al., 2001) have been compounded to enable owners to independently administer.

Palatability of oral formulations has been found to increase compliance level. Studies published in human medicine found that palatability is an important factor in drug compliance for children where the acceptability and ease of medication is greatly affected by its taste (Cifaldi et al., 2004; Cohen et al., 2009; Hames et al., 2008). The term “palatability” refers to the voluntary (free choice) acceptance of ingestion of a pharmaceutical composition by companion animals, which is measured by a standard palatability test; acceptance and preference testing (Litster et al., 2007; Thombre, 2004). Palatability is a desired attribute because it affects convenience and compliance, especially if medication has to be administered as a lifelong therapy e.g. given every day. Palatable oral formulations produced by pharmaceutical companies are commonly achieved by masking the taste and odour of drugs using chemicals, additives and flavourings (Thombre, 2004).

Other strategies can be adopted to minimise non-compliance by pet owners such as the clarity of instructions of prescription. They should be written clearly and backed up verbally to educate pet owners regarding the methods and intervals of administration. Changing a treatment regime from three times a day to twice daily and choosing a more appropriate dose or formulation would enhance compliance and effective treatment. Compliance levels also increase if owners are given more information regarding the condition being treated and the treatment provided if the veterinarian has spent sufficient time with pet owners during consultation (veterinarian-client-pet relationship) (Berendsen and Knol, 2002; Chapman, 1996; Cramer and Spilker, 1991; Grave and Tanem, 1999).

CONCLUSION

Oral formulation is a reliable method of administration. Currently, tablets at times have been substitute by paste and suspension formulation in clinical practice in doses equivalent to those given as tablets. To our knowledge, many studies has published reporting about specific drug plasma concentration and the pharmacodynamic effects but not many has looked at other formulations in comparison to tablet. Surveys or questionnaires documented to quantify owner preference and compliance towards different formulation of a drugs during administration are limited in veterinary medicine.

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