THE OCCURRENCE OF PRE-ANALYTICAL ERRORS IN DIAGNOSING OF PARASITE INFECTIONS IN DOGS AND CATS IN POLAND

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Summary. In veterinary practice, laboratory errors can be divided into three groups: pre-analytical, analytical and post-analytical errors. Pre-analytical errors can be subdivided into the errors occurring due to biological and non-biological factors. The aim of this study was to determine the types and the rate of occurrence for pre-analytical errors made by veterinarians in parasitological examinations of dogs and cats. The study was conducted in a private veterinary laboratory in Warsaw in the period Aug 2006 - Jul 2010. In total 7392 faecal samples, 371 skin scrapings and 43 parasite specimens or implied parasites for identification were collected. The results of the provided samples were archived with a note on the findings of pre-analytical errors or lack thereof. Overall errors were found in 6979 (89.4%) cover letters and 4459 (57.1%) samples out of 7806 delivered for examination. Pre-analytical errors detected in this study that were resulted from biological factors were not as prevalent as errors resulted from non-biological factors. The former errors resulted from ignorance of parasite biology. The latter group of errors indicates rather negligence, bad habit or lack of experience of veterinarians than ignorance.

Keywords: small animals, laboratory errors, laboratory practice, parasitological diagnostics, pre-analytical errors, veterinary parasitology.

KAČIŲ IR ŠUNŲ PARAZITINIŲ LIGŲ PREANALITINĖS DIAGNOZĖS YPATUMAI LENKIOJE

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Raktapoziciai: smulkiui gyvūnui, laboratorinė praktika, laboratorinės klaidos, parazitologinė diagnostika, preanalitinės klaidos, veterinarių parazitologija.

Introduction. Currently, veterinary diagnostic laboratory plays an important role in the diagnosis of disease, classification of animals to surgery with the use of general anaesthesia, the identification of zoonotic risk and in determining prognosis (Sirois, 2007). One of the important elements in the laboratory practice is parasitological diagnostics in which different developmental stages of parasites are identified. Feces, urine, skin scrapings, histopathological material, blood, the parasite specimens, or in case of flea infestation, their droppings can also be examined in parasitological examinations. It is important that samples delivered to the laboratory should be collected in sufficient quantity, suitably packed, stored and transported after properly preparing the animal for a specific
test. In *Giardia* sp. infection the number of samples tested is also important. It is recommended to collect at least three samples over a period 5 – 7 days to rule out *Giardia* infection (Zajac and Conboy, 2006). Moreover, it is important to accurately write cover letter supplied with the sample to be tested with indication of consignee clinic. In addition cover letter should contain the name of the owner of the animal, the animal's name (if any), age and species, the testing requested and in case of suspicion, the information on the presence of visible parasites or their fragments in the sample (Hendrix, 2007a; Taylor, 2007; Zajac and Conboy, 2006). Strict observance of pre-analytical procedures allows to reduce errors related to laboratory diagnostics.

In veterinary practice, laboratory errors can be divided into three groups of errors: pre-analytical, analytical and post-analytical errors. The latter ones are mainly associated with the recording of results, noted observations and interpretation of the results. Pre-analytical errors can be subdivided into errors due to biological and non-biological factors (Graber, 2006; Sirois, 2007). In parasitological diagnostics, the most common are the errors associated with the host (species, age and sex of the animal) and parasite (the development cycle, prepatent period). The errors resulting from non-biological variables can include errors associated with writing the cover letter, preparing the animal for the test, the method of sampling and sample number, as well as packaging, storage and transportation of samples. Both biological and non-biological factors may affect the proper diagnosis and further clinical investigation (Hendrix, 2007a; Sirois, 2007; Zajac and Conboy, 2006). It is also worth of noting that pre-analytical errors are the most common errors in laboratory diagnostics and the vast majority of them are made by clinicians or animal’s owner inadequately instructed by a clinician or a laboratory technician (Bonini et al., 2002; Lippi et al., 2010). However, there is a lack of data on the occurrence rate for pre-analytical errors in veterinary parasitological diagnostics in Eastern Europe, including Poland. Such errors can lead to further inappropriate investigations, resulting in an unjustifiable increase in costs, and also to inappropriate treatment or modification of therapy (Plebani and Carraro, 1997).

The aim of this study was therefore to determine the type and the occurrence rate for pre-analytical errors made by veterinarians in parasitological examinations of dogs and cats. It is expected that determination of the type and the frequency of errors made in parasitological diagnostics may help to reduce their occurrence in the future. This knowledge subsequently may help to improve the diagnostic process and may reduce the number of false results, and thereby increasing the credibility of both the laboratory and the veterinary practitioner.

**Materials and Methods.** The study was conducted in the period Aug 2006 - Jul 2010 in a private veterinary laboratory in Warsaw. In total 7392 faecal samples, 371 skin scrapings and 43 parasite specimens or implied parasites were collected (34 *Toxocara* sp., 9 tapeworm specimens or tapeworm proglottids, 2 non-biological objects). Out of 7392 faecal samples 3917 were collected from dogs, 3436 – from cats, and 39 samples – from unknown animal species. Five hundred and twenty seven samples were tested for the presence of *Giardia intestinalis* cysts, 719 samples were tested by flotation for the presence of gastro-intestinal parasites, while the rest of the samples were tested for both. In case of skin scraping tests, 322 out of 371 samples were collected from dogs and 28 samples – from cats. The rest of scrapings were collected from an unknown animal species. In latter case, animal species have been determined after additional consultation with clinics ordering the tests. It was revealed that among 39 unknown faecal samples 27 were collected from dogs and 12 from cats. In case of skin scraping tests all 21 samples with unidentified animal species were collected from dogs. In the case of parasite specimens provided for identification 20 were from dogs and 23 from cats.

Materials for analyses were delivered from 117 veterinary clinics in Warsaw and the surrounding area, and were delivered by laboratory employed couriers shipping the samples to the laboratory no longer than 2 hours. Faecal samples were transported in thermoses.

Samples submitted to the laboratory directly by animal owners and samples collected from horses, pigeons, budgies, rabbits, guinea pigs, gerbils, chinchillas, hampsters, rats, mice, ferrets and reptiles were excluded from the study. In addition, urine, vomit and scotch–tape samples collected from dogs and cats were excluded from the study due to insufficient number of those samples (11 urine, 1 of vomit and 14 scotch–tape samples).

The results of the delivered samples were archived with a note on the findings of pre-analytical errors or lack thereof. Examination of faecal samples collected from too young animals, examination of faecal samples for the presence of *Dipylidium caninum* eggs, examination of faecal samples collected from animals treated with paraffin oil within 24 hours before sample collection, too shallow scrape of the skin, too little material for testing, too few samples collected for faecal examination for the presence of *G. intestinalis*, tapeworm segments in formalin, improperly packaged scrapes of the skin (material between two microscope slides wrapped in a plaster bandage), extremely gasified faecal samples (probably kept at room temperature), and improperly written cover letters were considered as pre-analytical errors. Lack of information in cover letters such as: first and second name of the animal owner, animal species, age and name (or identification number), name of a veterinarian, name or stamp of a clinic, and description of the suspected object in faecal samples were considered as errors. Animal gender was considered as unimportant information for laboratory detection and identification of parasites in faeces or scrapings and for identification of an animal, clinic and owner of the animal. Therefore, unindicated gender was not considered as an error.

The results were analyzed using statistical package Statistica 8.0. Basic statistics such as arithmetical means, standard deviations (SD) and confidence intervals (CI) were calculated. Chi-square test was used to estimate the dependence of errors prevalence caused by biological and non-biological factors in faecal examinations.
**Results.** The occurrence of both type of pre-analytical errors resulting from biological agents and non-biological factors was recorded in this study. Out of 7806 samples delivered for testing the errors were found in 6979 cover letters and 4459 samples (237 skin scraping samples, 4219 faecal samples, 3 parasite specimens) (Table 1).

![Image of Table 1](image.png)

Table 1. The description and occurrence of pre-analytical errors

<table>
<thead>
<tr>
<th>Group of pre-analytical errors dependent on:</th>
<th>Kind of error</th>
<th>Error and prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biological factors</td>
<td></td>
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<tr>
<td>Ignorance of prepatent period</td>
<td>Examination of faecal samples collected from too young animals (younger than 1 week) in 11 out of 7392 (0.15%)</td>
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<tr>
<td>Ignorance of parasite life cycle</td>
<td>Examination of faecal samples for the presence of <em>D. caninum</em> eggs in 29 out of 7392 (0.39%)</td>
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<tr>
<td>Improper preparation of an animal for a test</td>
<td>Examination of faecal samples collected from animals treated with paraffin oil within 24 hours before sample collection in 4 out of 7392 (0.05%)</td>
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<tr>
<td>Improper sampling</td>
<td>Too shallow scrape of the skin (lack of blood and/or epidermal fragments) in 47 out of 371 samples (12.67%)</td>
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<tr>
<td>Non-biological factors</td>
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<tr>
<td>Improper preparation of the sample</td>
<td>Too little material for testing: – in 78 out of 7392 of fecal samples (1.05%) – in 40 out of 371 of scraping samples (10.78%)</td>
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<tr>
<td>Improper storage of the sample</td>
<td>Too few samples collected for fecal examination for the presence of <em>G. intestinalis</em> in 4011 out of 6673 (60.1%)</td>
<td></td>
</tr>
<tr>
<td>An improperly written cover letter</td>
<td>In total 6979 out of 7806 (89.40%) of cover letters written with missing data*: – Lack of 1 information in 4796 out of 6979 (68.72%) improperly written cover letters – Lack of 2 or more information in 2183 out of 6979 (31.28%) improperly written cover letters</td>
<td></td>
</tr>
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CI – confidence interval; *missing data – first and second name of the animal owner, species of animal, age of animal, name of animal, name of veterinarian, name or stamp of clinic, description of the suspected object in faecal samples.

In 87.06% (323/371; 95% CI 83.4% - 90.72%) of skin scraping tests one, two, three or four concurrent errors were recorded (Fig. 1). Arithmetical mean error number ± SD for all 371 skin scraping tests amounted to 1.717 ± 1.026. In 26.62% of skin scrapings with the errors (86/323; 95% CI 21.8% - 31.44%) one pre-analytical error (incomplete cover letter) was recorded. Two concurrent pre-analytical errors (incomplete cover letter + 190 improperly packaged skin scrapings or 2 too shallow skin scrapings) were recorded in 59.44% (192/323; 95% CI 54.09% - 64.79%) tests with errors (Fig. 2). Three concurrent errors (incomplete cover letters and too little material + 5 improperly packaged skin scrapings or 8 too shallow skin scrapings) were recorded in 4.02% (13/323; 95% CI 1.88% - 6.16%) tests with errors. Four concurrent errors (incomplete cover letters, too shallow and improperly packaged skin scrapings, and too little material for testing) were found in 9.91% (32/323; 95% CI 6.65% - 13.17%) tests with errors. Arithmetical mean error number ± SD for 323 skin scraping tests with pre-analytical errors amounted to 1.972 ± 0.839.

In 89.99% (6652/7392; 95% CI 89.31% - 90.67%) of faecal samples one, two or three concurrent errors were recorded (Fig. 3). Arithmetical mean error number ± SD for all 7392 faecal examinations amounted to 1.499 ± 0.718. In 37.07% of faecal samples with the errors (2466/6652; 95% CI 35.91% - 38.23%) one pre-analytical error (2433 improperly written cover letters or 22 examinations of faecal samples for the presence of *D. caninum* eggs or 11 samples of faeces collected from too young animals) was recorded. In 59.32% of faecal samples with the errors (3946/6652; 95% CI 58.14% - 60.50%) two concurrent pre-analytical errors (7 improperly written cover letters and examination of faecal samples for the presence of *D. caninum* eggs or 47 improperly written cover letters or 3775 improperly written cover letters and too few samples collected for faecal examination for the presence of *G. intestinalis* or 117 improperly written cover letters and extremely gasified faecal samples) were recorded. Three
concurrent errors (improperly written cover letters + too little material for testing and 31 too few samples collected for fecal examination for the presence of *G. intestinalis* or 205 extremely gasified faecal samples) were recorded in 3.55% (236/6652; 95% CI 3.11% - 3.99%). Arithmetical mean error number ± SD for 6652 faecal examinations with pre-analytical errors amounted to 1.665 ± 0.543.

In 86.05% (37/43; 95% CI 75.69% - 96.41%) of submitted parasites one or two concurrent errors were recorded (Fig. 4). Arithmetical mean error number ± SD for all 43 submitted parasites amounted to 0.930 ± 0.457. In 91.89% (34/37; 95% CI 83.09% - 100%) of submitted parasites with errors one pre-analytical error (only improperly written cover letter) was recorded. Two concurrent errors (improperly written cover letters and tapeworm segments in formalin) were recorded in 8.11% (3/37; 95% CI 0.69% - 16.91%). Arithmetical mean error number ± SD for 37 submitted parasites with pre-analytical errors amounted to 1.081 ± 0.277.

In 89.41% (6979/7806; 95% CI 88.73% - 90.09%) of cover letters attached to the samples one, two, three or four concurrent errors were found (Fig. 5). Arithmetical mean error number ± SD for all 7806 cover letters amounted to 1.232 ± 0.713. In 68.72% (4796/6979; 95% CI 67.63% - 69.81%) of improperly written cover letters lack of one datum (lack of an animal age in 2708 of letters or lack of name of a veterinarian in 2048 letters or lack of first name of the animal owner in 40 letters) was recorded. Lack of two data (such as: an animal and veterinarian name in 31 letters, an animal name and age in 465 letters, description of an object suspected to be a parasite and name of a veterinarian in 2 letters, name of a clinic and animal in 54 letters, an animal age and species in 2 letters, name of a veterinarian and clinic in 59 letters, name of a veterinarian and animal age in 422 letters, an animal age and first name of the animal owner in 702 letters) was recorded in 24.89% (1737/6979; 95% CI 23.88% - 25.90%). In 6.26% of improperly written cover letters (437/6979; 95% CI 5.69% - 6.83%) lack of three data (such as: an animal species and name of a clinic and veterinarian in 18 letters, an animal age and name and first name of the animal owner in 395 letters, first and second name of the animal owner and an animal age in 24 letters) was recorded.
letters) was recorded. Lack of four data (such as: first name of the animal owner and an animal name, age and species) was recorded in 0.13% of improperly written cover letters (9/6979; 95% CI 0.05% - 0.21%). Arithmetical mean error number ± SD for 6979 of improperly written cover letters amounted to 1.378 ± 0.607. Although lack of gender in cover letters was not considered as an error, it was noted that this datum was not written in 1217 letters. However, in most cases it was easy to predict the gender based on the name of an animal.

Chi-square test showed that there was significant dependence of errors prevalence caused by biological (11 cases of prepatent period ignorance + 29 cases of parasite life cycle ignorance out of 7392; 0.54% cases; 95% CI 0.37% - 0.71%) and non-biological factors (4 cases of improper preparation of an animal for a test + 78 cases of too little material collection for testing + 322 cases of improper storage of the samples out of 7392; 5.46% cases; 95% CI 4.94% - 5.98%) in faecal examinations ($\chi^2 = 307.65$, $p = 0.0000$).

Discussion. The results from this study showed that pre-analytical errors were dependent on non-biological factors and were the most prevalent in diagnosis of parasite infections in dogs and cats in Poland. An improperly written cover letter was the most common error for both the skin scrapings and faecal samples. Most of errors in these cover letters resulted from unindicated animal name or age, name of a veterinarian or first name of the animal owner. This type of errors caused some inconveniences with identification of the animal species. In particular, it was difficult to determine who is the owner of the animal when his first and second name was not indicated on the cover letter. In such cases, the animal owners were determined by phone call after consultation with the veterinary clinic providing the sample for examination. Another similar situations resulted from concurrent 4 errors in cover letters such as unindicated first name of the animal owner and animal name, age and animal species. In these cases consultation with the veterinary clinics were also required. However, such situations were not frequent.

Unindicated animal age in the cover letter was also considered as an error. In young animals it may be very important in what age faecal sample was collected. If collected during prepatent period the test result may usually be negative, whereas few days later parasite eggs may be excreted or adult helminths expelled in the faeces. To
prevent from such cases the laboratory should give the instructions on the best time for collection of samples (Hendrix, 2007a). The results from this study revealed that unindicated animal age was the most common error in the cover letters.

Fig. 3. Two concurrent pre-analytical errors in the skin scraping test – too shallow scrape of the skin (without blood and debris) and too little material for examination

Another important error observed in the cover letters in this study was missing description of parasite-like suspected object. In two cases the animal owner noticed the parasite-like object in the faeces, however the results of faecal examination were negative. The origin of parasite-like objects in the faeces was only determined after conversation with animal owner and re-examination of faecal samples. In first case the fragment of white string and in the second – styrofoam balls swallowed by the dog were detected. These two cases showed that description of parasite-like objects observed in faeces should be included by laboratory.

The errors in cover letters were probably due to the negligence or, in some cases, lack of experience. However, a large number of errors in the cover letters may also indicate on the low motivation or qualification of veterinarians.

Except from improper cover letters, improper preparation of the sample was the most prevalent pre-analytical error in the skin scraping tests. In these cases, material for examination was squeezed between two microscope slides and wrapped in a plaster bandage. Peeling off the plaster bandage from the slide or cutting the plaster bandage resulted in partial loss of tested material. After removing the plaster bandage, the slides were not suitable for further diagnosis due to presence of the residual glue on the surface (Fig. 2). This caused the sticking of slides to the microscope and concealed a large part of the observation field. Moreover, some debris and hairs stuck to the plaster bandage and this part of material was lost for testing. Thus, microscope slides wrapped in a plaster bandage is improper way of packing for skin scrapings. The properly prepared skin scraping samples were delivered in plastic tubes or placed between two slides wrapped in the paper. Both of these two ways were much better and did not cause any loss of tested material. Improper packing of skin scrapings is very important pre-analytical error because it causes loss of testing material which may lead to misdiagnosis, especially in case of low level of infection. Such cases may be observed when amount of material for testing is too small or scraping was too shallow (the skin scrapings without any blood) (Zajac and Conboy, 2006). However, these errors were not as prevalent as compared to those of improper preparation of the sample for delivery.

In this study the authors were unable to determine whether the scrapings were taken in the right place which may also influence on the efficacy of examination. Properly collected material should be taken from multiple sites and the sites selected for scraping should be at the periphery of a lesion (Hendrix, 2007b; Zajac and Conboy, 2006).

Since Giardia spp. cysts are excreted irregularly, three samples should be collected over a 5 to 7 day period in order to rule out the infection (Zajac and Conboy, 2006). Therefore collection of single faecal sample for examina-
tion was one of the most prevalent pre-analytical errors in the faecal tests. This kind of error may cause false negative results and lead to inappropriate treatment.

Another error which may lead to misdiagnosis in faecal tests was improper storage of the sample. Although this kind of data could not be collected in this study, more than 300 of faecal samples were extremely gasified. This may indicate on storage in too high temperature (in the room temperature at least). In good practice faecal samples should be examined within a few hours. If faeces cannot be examined within this time, the sample should be stored at +4°C to protect some nematode eggs from hatching within few days (Taylor et al., 2007; Zajac and Conboy, 2006).

Occasionally an important error was recorded, causing the complaints by client, grievances, and loss of confidence to a veterinarian or laboratory. In case of presence of proglottids of tapeworm *D. caninum* in the perianal area or animal faeces, the faecal flotation test was requested by veterinarian. The ignorance of reproducing behaviour for *D. caninum* where the eggs of this tapeworm typically are not excreted in the faeces (Taylor et al., 2007) always resulted in negative result. The eggs of *D. caninum* may be detected in faecal flotation tests only occasionally but in most of cases the result of the examination is negative (Bowman et al., 2002; Zajac and Conboy, 2006).

In few cases the animal was erroneously treated orally with paraffin oil before collection of faeces. This resulted in concealed observation field due to excess of oil in the sample leading to false negative result with subsequent re-examination after few days.

Fig. 4. Number of pre-analytical errors in faecal tests

(A – all samples, B – properly prepared samples, C – improperly written cover letter, D – too few samples collected for fecal examination for the presence of *G. intestinalis*, E – improper storage of the samples, F₁ and F₂ - examination of faecal samples for the presence of *D. caninum* eggs, G – too little material for testing, H – improper preparation of an animal for a test, I – ignorance of prepatent period).
Fig. 5. Number of pre-analytical errors in identification of a parasite

(A – all samples, B – properly prepared samples, C – improperly written cover letters, D – improper preparation of the sample).

Even is rear, the renouncement of examination due to lack of faecal material and due to request for faecal examination of 3- to 4-day-old dogs and cats was also recorded in this study. Examination of such young animals cannot be substantiated as the shortest prepatent period for gastro-intestinal parasites is significantly longer than 4 days (Bowman et al., 2002; Hendrix, 2007a; Taylor et al., 2007). Moreover, the infection with intestinal coccidian protozoans, having the shortest prepatent period, in most of cases is acquired by eating the tissues of intermediate or paratenic hosts (Taylor et al., 2007). Thus, detection of oocysts or parasite eggs in faeces is impossible in such young age. These two types of errors resulted from ignorance of prepatent period for parasite infections and from ignorance of minimal amount of 5 grams of faeces required for examination (Taylor et al., 2007).

In this study the authors also found few cases with error resulted from improper preparation of a parasite sample for examination. This error was sending of tapeworm segments in formalin. Based on the shape of proglottids they were probably D. caninum segments. However, it was impossible to identify the segments since formalin dehydrated the segments leading to multiply fractures during compression between two slides. This was considered as an error because identification of D. caninum segments is based on visibility of characteristic two sets of genital organs with separate openings on each side or released egg packets. Compression of gravid segments with small amount of tape water allow to demonstrate egg packets and two sets of genital organs in the segment (Bowman et al., 2002; Zajac and Conboy, 2006).

Pre-analytical errors resulted from ignorance of parasite biology were not as prevalent when compared to those resulted from non-biological factors in this study. The high occurrence of latter group of errors may indicate on negligence, wrong habit or lack of experience by veterinarians.

This study showed that pre-analytical errors are important factors that may interfere with results of the tests and therefore are underestimated in the veterinary practice. Similar studies from the field of laboratory medicine showed that the error rate ranges from 0.1 to 3.0% of laboratory results (Lippi et al., 2010). Plebani and Carraro (1997) reported that 68.2% of laboratory errors originated in the pre-analytical phase, compared with 18.5% in the post-analytical phase. The rest of laboratory errors...
(13.3%) originated from the analytical phase. Comparison of the results of this study with the results observed in laboratory medicine showed lack of standards and discipline in preparing samples for testing in the field of veterinary practice. Moreover, it shows that a lot of veterinarians probably are not aware of the existence of pre-analytical errors. It seems that the most important factor that may affect the error rate reduction is good communication between laboratory and clinic. Similar conclusions were also made by physicians (Plebani, 2006). In the authors’ opinion, reducing errors in the cover letter may also affect the form of it. It could be concluded that improving the design of the cover letter may reduce some errors. Many pre-analytical errors may be also reduced by education of veterinarians in the field of laboratory procedure. As earlier suggested by Plebani (2006), it is also important for veterinarians to better understand the principles of operation in veterinary clinical laboratory. It is important to understand on how the tests are performed, which test is appropriate for particular parasitosis, and which factors are responsible for false results. It seems probable that this knowledge may help to reduce pre-analytical errors in veterinary parasitological diagnostics.

Fig. 6. Number of pre-analytical errors in cover letters

(A – all cover letters, B – properly written cover letters, C – improperly written cover letters, D – lack of first name of the animal owner, E – lack of second name of the animal owner, F1, F2, F3 – lack of animal species, G – lack of animal age, H1, H2 – lack of animal name, I – lack of name of a veterinarian, J1, J2 – lack of name of a clinic, K – lack of description of an object suspected to be a parasite).

Acknowledgements
The authors would like to thank staff at the Veterinary Diagnostic Laboratory Lab-wet in Warsaw for their cooperation and assistance in collection of samples.

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Received 22 November 2010
Accepted 12 May 2011