PREVALENCE OF ANTIBODIES TO BVDV AND BHV1 IN DAIRY HERDS IN SOUTHEASTERN REGION OF ROMANIA

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Summary

Bovine viral diarrhoea virus (BVDV) and infectious bovine rhinotracheitis virus (IBRV) are important viral diseases around the world. The objective of this study was to estimate the prevalence of seroconversion to BVDV and IBRV. Serum samples from 152 bovines were collected from two counties (Tulcea and Brăila) in the southeastern region of Romania. The samples were tested for presence of antibodies to BVDV and BHV-1 using two commercial indirect ELISA kits. The results showed that seroprevalence of infectious bovine rhinotracheitis (IBR) was 29.6% and that of bovine viral diarrhea (BVD) was 41.44% in tested cows. Antibodies to both viruses were found in only 24 tested cattle, representing a prevalence of 22.36%. The presence of BHV1 antibodies was associated with a higher prevalence of bovine viral diarrhoea virus (BVDV) antibodies.

The relatively high incidence of seroconversion for BVDV suggests that detailed knowledge of the epidemiology of bovine virus diarrhoea is essential for identification and elimination of PI animals. The scenario of IBRV is favorable to implement a program directed to culling seropositive cows and not keeping infected heifer calves.

Key words: Infectious bovine rhinotracheitis, bovine viral diarrhea, seroprevalence

The main infectious agents involved in bovine respiratory disease in Romania are viruses and bacteria. Antibiotics can be used to combat bacteria but are ineffective at fighting viruses. Those viruses and bacteria most commonly linked with bovine respiratory disease are infectious bovine rhinotracheitis virus (IBR) and bovine viral diarrhea virus (BVD).

Bovine herpesvirus 1 (BHV1), the causative agent of infectious bovine rhinotracheitis (IBR), is considered to be the most common viral pathogen found in bovines. BHV1 is associated with several clinical conditions including infectious bovine rhinotracheitis, infectious pustular vulvovaginitis, balanoposthitis, conjunctivitis and generalized disease in newborn calves causing great economical losses (8). Like the other alphaherpesviruses, BHV-1 can establish a latent state in ganglionic neurons after infection (4). BHV-1 can be reactivated and reexcreted by means of several stimuli, including transport, parturition, and treatment with glucocorticoids (11). Latency allows the virus to persist, and the introduction of a latently infected carrier into a non-infected herd is the best way to spread the disease (2).

Bovine viral diarrhea virus (BVDV) is a ubiquitous pathogen that infects domestic animals such as cattle, goats, sheep, and pigs, as well as many wild and captive animals. Bovine viral diarrhea virus is an important infectious disease agent...
of cattle that can potentially have a negative effect on all phases of reproduction (9). Depending on the stage of gestation, these can include failure to conceive, early embryonic loss, abortion, mummified foetuses, congenital defects and the birth of persistently infected (PI) calves. While acute/transient BVDV infections that may be inapparent or that may cause respiratory and digestive tract disease can lead to BVDV transmission among a group of cattle, many believe that the PI calf is the key to sustaining the virus in a population of cattle (7).

The hazards associated with these infections are also well recognised. Acute infection of naive immunocompetent cattle with BVDV can have a variety of negative outcomes. In calves in particular it can induce a pronounced immunosuppression, which is known to potentiate the effects of concurrent infection with a wide range of bacterial and viral pathogens, including those that cause respiratory and enteric disease. In older animals associations have also been shown with reduced milk yield, increased risk of clinical mastitis and retained foetal membranes and increased somatic cell counts.

BVDV have four major structural antigenic polypeptides. Glycoprotein E causes production of significant levels of antibodies in animals. Antibodies to E have limited neutralizing activity. Glycoprotein E2 is antigenic targeted for antibodies. E2 is highly antigenic the production of neutralizing antibodies in the host after infection (5). Nonstructural protein 23 has two separate polypeptides – NS2 and NS3. Protein NS3 is marker of citopatic BVDV and is the most conserved protein in the pestivirus family. This polypeptide is very stable in infected cells and highly immunogenic (6). The ability of BVDV antibodies to protect (neutralizing) against BVDV infection is dependent on the virus strain, level of antibody produced. An indirect measure of virus infection is the detection of virus-specific antibodies in the sera of animals. Many tests are available for the detection of anti-BVDV antibodies (10).

Materials and methods

Sera from 152 home-bred cattle were collected from two counties. All blood samples were obtained in cluster haphazard sampling from apparently healthy cattle in ten localities from Brăila and eight localities from Tulcea County. All specimens surveyed in this study were collected from September to December 2012. The sera were separated from blood samples after centrifugation 10 minutes at 1500 rpm and were stored at –20°C until use.

Serum samples were both tested for the presence of antibodies to glycoprotein B (gB) BHV-1 and BVDV, using two commercially available ELISA: HerdChek® IBR gB Blocking Ab Test and HerdChek® BVDV Total Ab Test, IDEXX, France. The IBR-gB enzyme immunoassay is based on the detection of specific antibodies to the BoHV-1 glycoprotein B (gB), meanwhile the BVDV antibody kit detects antibodies against the non structural proteins NS3 of BVDV.

The protocol was made according to the manufacturer’s recommendation. Briefly, the antibody detection kits required the over-night sera incubation in
antigen–coated wells. After washing unbound materials, a specific peroxidase-conjugated monoclonal antibody was added for being detected with substrate/chromogen solution. The absorbance value was measured at a single wavelength of 450 nm and the results were expressed as optical density (OD).

**Results and discussions**

Several studies worldwide carried out to evaluate BHV-1 or BVDV seroprevalence, but very few studies evaluate the distribution of both infection contemporaneously. The serological survey carried out in two counties: Brăila and Tulcea revealed that 24 (22.36%) out of 152 home-bred cattle were contemporaneously seropositive for BHV-1 and BVDV antibodies.

In Brăila county twenty-seven serums out of 77 tested were found positive for antibodies to glycoprotein B (gB) BHV-1, representing a seroprevalence of 35.06%. In addition, 37 (48.05%) out of 77 animals tested were positive for BVDV antibodies.

<table>
<thead>
<tr>
<th>Brăila County localities</th>
<th>No. of samples tested</th>
<th>No. of positive samples for BHV-1 antibodies</th>
<th>No. of positive samples for BVDV antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ianca</td>
<td>7</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Semenele</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mărășu</td>
<td>8</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>Salcia-Tudor</td>
<td>8</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Grădiștea</td>
<td>8</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>Cazasu</td>
<td>8</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Stâncuța</td>
<td>6</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Movila miresii</td>
<td>8</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Roșiori</td>
<td>8</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Vlădeni</td>
<td>8</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>77</strong></td>
<td><strong>27</strong></td>
<td><strong>37</strong></td>
</tr>
</tbody>
</table>

In this county out of 77 samples tested eighteen (23.37%) were found positive for both types of antibodies.

The percentage of samples having antibody to BHV1 varied from 100% to 0%. Higher and lower percentages of positives were reported from Grădiștea and Ianca/Semenele, respectively. Regarding the BVDV antibodies, the percentage varied from 87.5% to 0%. Higher percentages of positives were reported from Grădiștea, Mărășu and Salcia-Tudor (7 out of 8).

From Tulcea County were tested 75 home-bred cattle in eight localities. The percent of positivity for BHV-1 antibodies was 24% (18 out of 75) and for BVDV antibodies was 34.66% (26 out of 75). Out of seventy-five home-bred cows tested only six (8%) were found seropositive for both antibodies. The greatest
percentages of seropositivity for antibodies anti-BHV1 was registered in localities: Cerna (45.5%) and Dorobanțu (37.5%).

<table>
<thead>
<tr>
<th>Tulcea County localities</th>
<th>No. of samples tested</th>
<th>No. of positive samples for BHV-1 antibodies</th>
<th>No. of positive samples for BVDV antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Niculițel</td>
<td>12</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Cerna</td>
<td>11</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>IC Brătianu</td>
<td>9</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Smârdan</td>
<td>8</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Isaccea</td>
<td>9</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Horia</td>
<td>9</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Dorobanțu</td>
<td>8</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Nalbant</td>
<td>9</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>75</strong></td>
<td><strong>18</strong></td>
<td><strong>26</strong></td>
</tr>
</tbody>
</table>

The values of seropositivity for BVDV antibodies were ranging from 50% in Niculițel and Dorobanțu, to 11.11% in Horia.

There was no correlation between the serological results and any evidence of clinical disease in cows. Note that we assume that antibody positivity remains for life after infection; if antibody titer wanes with time since infection, then a conversion from positive to negative might reflect a true status rather than a test failure. The serological tests used in the current study did not distinguish between vaccine derived and pathogen derived antibody so this was unavoidable.

Testing a serum sample for IBR antibodies will indicate the IBR status and whether cattle are most at risk from acute or chronic IBR infection. The interaction of BVDV with the immune system is unique among viruses. The modulation of the immune system by BVDV strains rarely kills its host but allows that virus to replicate and evolve. In this sense it is a very successful pathogen that is able to replicate and establish persistent infections. Seroepidemiological survey results should be viewed with some reservation because of the PI animals (persistently infected), that give negative serological response for BVD-MD.

**Conclusions**

In the present study was considered to perform serological investigations regarding two viruses that produce two morbid entities namely: infectious bovine rhinotracheitis (IBR) and bovine viral diarrhea-mucosal disease (BVD) considering them the main causes of respiratory diseases that occur in Romania. Today many countries are trying to apply surveillance and control programs for these diseases. These programs consist of a very strict control of animal movements, the fulfillment of biosecurity measures and serological and etiological surveillance.
Acknowledgments

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References

PRELIMINARY RESULTS REGARDING THE PREVALENCE OF
TOXOPLASMA GONDII IN DIAPHRAGM TISSUE FROM
BACKYARD PIGS IN CENTRAL ROMANIA

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Summary

The aim of this study was to evaluate the prevalence of Toxoplasma gondii DNA in
diaphragm tissue from backyard pigs, by conventional PCR. Sixty diaphragm tissues were
collected from pigs, reared in backyard system, from four counties (15 samples per county)
located in central Romania (Alba, Cluj, Hunedoara and Mureş). T. gondii DNA in the
samples was evaluated by PCR technique, using specific primers located on the B1 gene,
designed to specifically amplify a portion of the ITS-1 region. The overall prevalence of T.
gondii DNA in diaphragm tissue was 8.33% (5/60). The highest prevalence was obtained in
Mureş county (20%; 3/15) followed by Alba and Cluj counties (6.7%; 1/15), while in
Hunedoara county none of the samples presented T. gondii DNA. The prevalence obtained
in our study showed that pigs reared for familial consumption in backyard system, can be a
source of human infection with T. gondii.

Key words: Toxoplasma gondii, PCR, diaphragm, domestic pigs.

Toxoplasmosis is one of the most important foodborne zoonosis. Pigs are
important to the economy of many countries because they represent a source of
food for humans, Romania being known as one of the countries where pork
consumption is relatively high. Most pigs acquire T. gondii infection postnatally by
ingestion of oocysts from contaminated environment or ingestion of infected
tissues, especially of rodents (4). Consumption of raw or uncooked meat,
especially pork, is a major risk factor of foodborne transmission for humans,
infected pork being an important source of T. gondii infection in humans (6).

The aim of this study was to evaluate the prevalence of T. gondii in
diaphragm tissue from backyard pigs from different counties of central Romania
and to estimate the potential risk of human contamination.

Materials and methods

Animals and samples

In December 2012, 60 diaphragm tissue samples from pigs, reared in
backyard system, from 4 counties of central Romania (Alba, Cluj, Hunedoara and
Mureş), were collected. All tested pigs were reared for familial consumption. For each county, 15 samples were studied. The samples were stored at -20°C until use.

Polymerase chain reaction

All the samples were tested by conventional PCR. DNA was extracted from 40 mg diaphragm tissue using a commercial kit (Bioline, UK), according to the manufacturer’s protocol. PCR was performed on all samples to detect *T. gondii* DNA. Two specific primers Tox4 (5’- CGCTGCAGGGAGGAAGACGAAAGTTG - 3’) and Tox5 (5’- CGCTGCAGACACAGTGTCATCTGGATT - 3’) (Generi-Biotech, Czech Republic) were used to amplify a 529 bp fragment. PCR amplification was performed in 25 μl reaction mixture consisted of 12.5 μl of MyTaq Red HS Mix (Bioline, UK), 25 pM of each primer (Tox4 and Tox 5) and 4 μl of DNA. The reaction mixture was initially incubated at 95°C for 1 min, followed by 35 amplification cycles of 15 s at 95°C, 15 s at 60°C, 10 s at 72°C and 5 min at 72°C. Aliquots of each PCR product were electrophoresed on 1.5% agarose gel and visualized under UV light (BIO DOC-ITM Imagine System). DNA fragment size was compared with a standard molecular weight (100 bp DNA ladder – Fermentas). RH strain was used as positive control.

Statistical analysis

Frequency, prevalence and its 95% confidence interval of *T. gondii* infection were established. A *p* value of <0.05 was statistically significant. All statistics were performed using the EpiInfo 2000 software.

Results and discussions

PCR result and prevalence of *T. gondii* DNA in backyard pigs from central Romania are presented in Fig. 1 and Table 1. The overall prevalence of *T. gondii* DNA in diaphragm tissue was 8.33% (5/60). The highest prevalence was obtained in Mureş county (20%; 3/15) followed by Alba and Cluj counties (6.7%; 1/15), while in Hunedoara county none of the samples presented *T. gondii* DNA.

The aim of this study was to evaluate the prevalence of *T. gondii* DNA in diaphragm tissue from backyard pigs in central Romania, by conventional PCR and to estimate the potential risk of human contamination by consumption of raw or undercooked infected pork.

PCR is a sensitive, highly specific and rapid technique (12) that is used to detect infections with *T. gondii*. Our data showed that a significant proportion (8.33%) of pork was contaminated with *T. gondii* based on PCR results.

Considering the fact that only 40mg of diaphragm tissue were examined by PCR technique, a negative result do not necessary mean that the whole tissue is *Toxoplasma*-free. Aspinall et al. (2002) concluded that the risk of human infection may not be expressed by PCR. Moreover, seropidemiological studies in Romania have showed that the prevalence of *T. gondii* in backyard pigs varies from 5.4% to...
87.9% (3, 7, 10). This variation is probably related with the raising and feeding system.

### Table 1

<table>
<thead>
<tr>
<th>County</th>
<th>Frequency</th>
<th>Prevalence %</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alba</td>
<td>1</td>
<td>6.67</td>
<td>0.17-31.95</td>
</tr>
<tr>
<td>Cluj</td>
<td>1</td>
<td>6.67</td>
<td>0.17-31.95</td>
</tr>
<tr>
<td>Mures</td>
<td>3</td>
<td>20</td>
<td>4.33-48.09</td>
</tr>
<tr>
<td>Hunedoara</td>
<td>0</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>TOTAL</td>
<td>5</td>
<td>8.33</td>
<td>2.76-18.39</td>
</tr>
</tbody>
</table>

**Legend:** CI – confidence interval

![Analysis of PCR products by agarose gel electrophoresis.](image)

Fig. 1. Analysis of PCR products by agarose gel electrophoresis. PCR was based on the amplification of a 529 bp fragment. Lane 1 – molecular weight markers, lane 2 – positive control, and lanes 3, 5, 6, 7, 8, 9 – positive results.

It has been shown that pig’s diaphragm frequently contains parasite cysts (5). In Romania, Pop et al. (1989) bioassayed diaphragms of 740 pigs from slaughterhouses and isolated viable *T. gondii* from a higher percentage of pigs (7 of 74 swine pools; 10%) than we detected by PCR. Other studies made worldwide indicated a higher prevalence of *T. gondii* in diaphragm tissue (34%) by PCR (2).

The most important risk factor for infection with *T. gondii* in backyard pigs, indicated by our data, is linked to the environmental exposure. Generally, in Romania, backyard pigs are reared in the presence of cats, which are shedding oocysts into the environment, and rodents, which have an important role as intermediate hosts in toxoplasmosis (4, 10). The environmentally resistant *T. gondii*-like oocysts in Romanian cats were detected in 1.2% of samples (9). Iovu (2011) detected *T. gondii* DNA in 4.7% of samples from cat feces.

Data obtained in our study showed that *T. gondii* infection has a public health impact. The food safety problems associated with the consumption of...
infected pork and the high level of environmental contamination indicate a direct health risk for humans from the studied area. It is important to improve the rodent control and the contact with cats to eliminate the possible risk of swine contamination.

Conclusions

The prevalence of *T. gondii* infection in diaphragm tissue from backyard pigs in central Romania was 8.33%.

The high prevalence of *T. gondii* infection in backyard pigs indicate that raw or undercooked pork is a possible important source of human toxoplasmosis.

Acknowledgements

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DETECTION OF BOVINE PAPILLOMAVIRUS TYPE 2 IN CUTANEOUS FIBROPAPILLOMAS IN CATTLE

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Summary

Bovine papillomavirus type 2 (BPV-2) is associated with the development of hyper-proliferative lesions of the epithelial and dermal cells and urinary bladder tumors in cows. In cattle, one of the consequences of the infection with this virus is the occurrence of cutaneous warts, which can be responsible for significant economic damages due to the retarded growth of the animals, loss of weight and decrease in milk production. The aim of this study was to detect by PCR the presence of BPV-2 DNA in cutaneous fibropapillomas and normal skin samples. Four skin samples from healthy cows and ten bovine cutaneous fibropapillomas were tested by PCR using two sets of primers for the amplification of BPV-2 E2 and E5 genes. BPV-2 E5 DNA was amplified in all tested fibropapilloma samples (100%) and in three out of four normal skin samples, while the DNA of BPV-2 E2 was amplified in five out of ten fibropapilloma samples (50%) and in two out of four normal skin samples (50%). Consequently, the sequencing of the amplicons confirmed the presence of BPV-2 DNA in the tested samples. These results confirm the efficacy of PCR targeting BPV-2 E5 gene and reveals the importance of BPV epidemiological studies in apparently healthy and papillomatosis-affected cattle to understand the spreading of this virus even in apparently healthy cattle.

Key words: Bovine fibropapilloma; BPV-2; PCR

Papillomaviruses (PV) are classified in Papillomaviridae family, divided in 16 genera. Generally, these viruses are causing hyper-proliferative lesions of the epithelial and dermal cells in humans and animals (5). Specifically, the lesions induced by PV are benign and usually with spontaneously regressing, although some lesions can undergo neoplastic transformation under the influence of environmental co-factors, such as consumption of the fern (3). PV are species-specific, the only case of cross-infection is noted on Bovine papillomavirus (BPV) type 1 and 2, which can infect the horses, mules and donkeys (7). BPV-1 and -2 are classified in the Deltapapillomavirus genera along with BPV-13 (6) and are
causing cutaneous and mucosal fibropapillomas in cattle (4). The genome of BPV-1 and -2 contains a double stranded DNA with 7900 bp, divided in early (E1, E2, E4, E5, E6, E7 genes) and late (L1 and L2 genes) (8).

In cattle, one of the consequences of the infection with this virus is the occurrence of cutaneous warts, which can be responsible for significant economic damages due to the retarded growth of the animals, loss of weight and decrease in milk production. Although bovine cutaneous warts are occurring in cattle of different age and breed from Romania, there are no studies concerning the detection of the BPV involved in the occurrence of the bovine cutaneous fibropapillomas.

The aim of this study was to detect the presence of BPV-2 DNA in cutaneous fibropapillomas and normal skin in cattle by PCR using two different sets of primers.

**Materials and methods**

Four normal skin samples and ten bovine cutaneous fibropapillomas were collected from cows of different breeds and ranging in age between 6 months and two years. The samples were immediately snap frozen in liquid azote and then conditioned at -80°C until the molecular analysis. The DNA was extracted from the fibropapillomas F1-F10 and from skin samples S1-S4 using the Dneasy Blood and Tissue kit (Qiagen), according to manufacturer instructions.

PCR for the detection of BPV-2 DNA were used two sets of distinct primers: one set for the detection of BPV-2 E5 gene, nucleotides 3842-3995 and the second set for the detection of BPV-2 E2 gene, nucleotides 3726-3801 (table 1).

<table>
<thead>
<tr>
<th>PRIMERS</th>
<th>REGION</th>
<th>SEQUENCES/SIZE</th>
</tr>
</thead>
<tbody>
<tr>
<td>BPV 2</td>
<td>E5 GENE</td>
<td>3842-3995 (153 BP) F – 5’ CAC TGC CAT TTG TTT TTT TC 3’ R – 5’ GGA GCA CTC AAA ATG AAT CC 3’</td>
</tr>
<tr>
<td>BPV 2</td>
<td>E2 GENE</td>
<td>3726-3801 (76 BP) F – 5’ GGC ACA GAT CTT GAT CAC CTT 3’ R – 5’ TCC AGG AGG TAG TGG GAC AT 3’</td>
</tr>
</tbody>
</table>

The amplification was conducted in a final volume of 50 μL, containing 10 μL of sample DNA, 3 mM MgCl2, 0.02 U/μL Platinum Taq (Invitrogen), 0.5 pmol/μL each oligonucleotide primer, 200 μM each dNTP. For the detection of the BPV-2 E5 gene the conditions were denaturation for 3 minutes at 95 °C, followed by 35 cycles of denaturation at 95 °C for 45 seconds, annealing at 50 °C for 45 seconds and extension at 72 °C for 1 minute, with a final extension for 5 minutes at 72 °C. For detection of BPV-2 E2 gene, the PCR cycle consisted of an initial denaturation
at 95 °C for 3 minutes, followed by 35 cycles of denaturation at 95 °C for 45 seconds, annealing at 52 °C for 40 seconds and a final extension at 72 °C for 40 seconds. Subsequently, the PCR products were separated by electrophoresis in 2% agarose gels with Tris acetate ethylene diamine tetraacetic acid (EDTA) buffer (TAE; 40 mM Tris, 1 mM Na₂EDTA, 20 mM acetic acid), at a constant voltage (100 V) for approximately 35 minutes, then stained with ethidium bromide and visualised under ultraviolet light. The PCR products E5 and E2 were purified using the Wizard SV Gel and PCR Clean-Up System Kit (Promega) and directly sequenced using the ABI Prism 3100 Genetic Analyzer (Applied Biosystems).

**Results and discussions**

BPV-2 E5 DNA was amplified in all tested fibropapilloma samples (100%) and in 3 out of 4 normal skin samples (figure 1), while the DNA of BPV-E2 was amplified in 5 out of 10 fibropapilloma samples (50%) and in 2 out of 4 normal skin samples (figure 2). Amplicons were sequenced, confirming the presence BPV-2 DNA.

**Table 2**

<table>
<thead>
<tr>
<th>SAMPLES</th>
<th>BPV-2 E5</th>
<th>BPV-2 E2</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>F2</td>
<td>+</td>
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</tr>
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<td>F3</td>
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<tr>
<td>S4</td>
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</tbody>
</table>

Legend: F1-F10 fibropapilloma samples; S1-S4 normal skin samples; + positive samples; - negative samples.
In accordance with other authors (9, 10), in this study was identified the presence of BPV-2 DNA both in normal skin samples collected from healthy cows and fibropapillomas. Using these type specific sets of primers, PCR assay may be a successful method to detect BPV DNA, but its sensibility and specificity may be affected by the concentration and the purity of sample DNA. Moreover, the DNA of human papillomavirus was detected by PCR assay in healthy skin, suggesting a subclinical or latent infection with HPVs (1, 2).

Conclusions

These results confirm the efficacy of PCR targeting BPV-2 E5 gene and reveals the importance of BPV epidemiological studies in apparently healthy and papillomatosis-affected cattle to understand the spreading of this virus even in apparently healthy cattle.
Acknowledgments

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References

INVESTIGATIONS REGARDING THE VALUE OF SOME DIRECT AND INDIRECT DIAGNOSE TESTS FOR CATTLE SUBCLINICAL MASTITIS

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Summary

For this study were carried out several tests of cow mastitis investigation – the most well known – which may lead to a presumptive diagnosis of clinical or subclinical mastitis, comparative evaluations, and with an emphasis on the value of cytological tests.

The main goal of the research subject was to establish, precisely, the value of the advantages and disadvantages of cytological test (milk cytogram) in compare with other diagnostic tests, such as: R-Mastitest, electrical conductivity, somatic cell count and bacteriological exam. Also, in order to achieve our main goal, we will be able to establish the minimum number of indirect and direct examinations, which may indicate, with certainty, the installation of an infectious process in the mammary gland.

Key words: mastitis, diagnosis, milk cytogram, investigations

The installation and the evolution of cattle mastitis affect the quality and quantity of milk products and represent a potential risk factor for other cows from the same habitat. In a well managed farm, subclinical mastitis, together with the clinical cases, should be effectively and rapidly detected.

Any mastitis type can produce changes in the composition of the milk, but the magnitude and variety of them depend on the inflammatory response induced (6). In other words, changes in the composition of milk secretion depend on the pathogenicity of bacteria and on the degree of deterioration in the mammary gland tissue, especially if they include alveolar epithelial secretory cells.

The main changes which are found in the mammary gland, following the installation of infectious and inflammatory processes, include:

- increased vascular permeability, which is followed by the penetration of protein enzymes and ions from the blood into the milk;
- massive influx of phagocytes, which penetrate the mammary glandular cisterns;
- decreased secretory ability of alveolar epithelial cells, manifested by decreasing the concentration of certain constituents of milk;
- presence of substances produced as a result of inflammatory reactions, such as acute phase proteins in affected quarter`s milk (3, 7).
Some compositional changes of milk are much more significant than others, but all are particularly useful in the diagnosis of mastitis.

Therefore, at the present time, the criteria for the most important investigation, which form the basis of a clinical laboratory for the diagnosis of mastitis in cows, have in mind:

- the number and type of pathogenic micro-organisms, which are present in milk and mammary gland;
- increasing the number of somatic cells in the milk, which reflects the infection – the host defense response against microbial agents;
- quantitative and qualitative changes of milk constituents, which reflects the deterioration or destruction of alveolar epithelial cells (5).

The diagnosis of clinical and subclinical Mastitis does not mean anything other than signs and quantify of these changes, which can be done by laboratory methods and techniques, or expeditiously, on stables or milking stand.

The standard method for measurement of mammary gland inflammation is represented by the cytological investigation, which includes the determination of the total number of somatic cells in the milk or other techniques associated with it. Mastitis diagnosis, in accordance to the recommendations of the International Dairy Federation (IDF), is based on two principles: the determination of the total number of somatic cells in the milk and the microbiological status of the udder quarters. In the present, the international scientific community, still try to establish diagnostic criteria f mastitis, and especially the minimum thresholds at which it is considered the infection and inflammation in the mammary gland. Some of the landmark suggestions are presented in table 1 (2, 4, 10):

<table>
<thead>
<tr>
<th>RESULTS OF BACTERIOLOGICAL AND CYTOLOGICAL EXAMS</th>
<th>DIAGNOSIS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>The presence of bacteria</strong></td>
<td><strong>Number of milk somatic cells ( higher the 200000 cells / ml of milk)</strong></td>
</tr>
<tr>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

\(^{1}\) after International Dairy Federation
\(^{2}\) after National Veterinary and Research Institute, Finland

To determine the total number of somatic cells in the milk, which is a standard method of diagnosis of mastitis, applying the techniques of analysis poses a problem when it comes to establishing them as routine examination. In addition,
bacteriological exam, although it is sovereign in mastitis diagnosis, cannot be applied as a routine test because of the high cost and time of execution. Therefore, the application of indirect tests of mastitis diagnosis remains preferable method, at least in the first phase, the milk of identified affected cows to be then examined by bacteriological procedures.

According to data from the literature, the infection installation in the cows mammary gland is manifested by an increase in electrical conductivity of milk, in the local temperature, in the number of somatic cells and others. Most of the times, the host response to the action of bacterial agents leads to self-sterilization and healing, without any therapeutic intervention. However, when the mammary gland infection does not heals by itself, through its own immune defense mechanisms, this becomes a pathological problem (1, 8, 9).

Taking into account the facts presented above, a unique and simple measurement of a certain parameter of milk (conductivity, pH, somatic cell count, enzymes and others) may not indicate and can not make the difference between mammary gland infection, immediately followed by healing, against the infection that occurs in a row after a breaking down of the immune defense mechanisms.

Materials and methods

In our country, mastitis of dairy cows are particularly frequent, causing major losses for both growth and operating farms and, for this reason, it is necessary to adopt consistent strategies for prevention and control. Without denying the fact that therapy with antibiotics is one of the essential points of these strategies, it is known that it doesn't always corrects the epidemiological situation, because, more often than not, is applied improperly, that is carried out in the absence of exact data concerning the nature and its causative microbial agents of mastitis. This explains, in part, why there are failures related to treatments applied are not a rarity, and why many cows may show several episodes of acute clinical mastitis.

By virtue of the well-known medical doctrine, according to which prevention is more effective than therapy, avoiding particularly large losses cause by cows mastitis, it would be possible to a great extent, through the establishment in each farm, of a complex program, comprising, in addition to strict rules of hygiene and the correct clinical mastitis therapy cast, a careful monitoring of the occurrence and development of subclinical mastitis, followed by concrete measures.

In order to achieve our objective of investigations, as well as the establishing the minimum number of indirect and direct examinations, which may indicate, with certainty, the installation of an infectious process in the mammary gland, has opted for the use of five comparative diagnostic tests for mastitis identification.

These indirect and direct tests are presented in chronological order of use, as follows: R-Mastitest, Mast-O-Test (for electric conductivity), bacteriologic exam (BE), milk cytogam (Neutrophils %) and somatic cells count (SCC).
For this study were investigated 76 cows (304 quarters, respectively) by applying the first two indirect tests: R-Mastistest (R-M) and Mast-O-Test (M-O-T). From those 76 cows, a number of 17 cows (68 quarters) were investigated using all the above mentioned tests. The reasons that we stopped the study at only 68 milk samples analyzed by all tests, were objectives: very high individual work volume in a very short time (to avoid cellular changes in the mammary gland, which would have constituted an impediment to the interpretation of data) and very high costs for kits and reagents.

Milk samples examined by the two indirect tests (R – Mastitist, Mast-O-Test) have been executed at the stable line, in a farmhouse from Timiș County.

The other three tests (bacteriological, cytological examination and counting of milk somatic cells in the ) were carried out in the laboratories of the Faculty of Veterinary Medicine Timişoara.

The data obtained as a result of our investigations were interpreted statistically using CORREL software program.

Next, to find out which of the tests may be associated most closely in order to establish the diagnosis of mastitis, we computed the correlation coefficient (i.e. numerical index which gives a measure of the relationship between two discrete or continuous quantitative variables), without being tied to the sensitivity or specificity of tests investigated.

Results and discussions

This correlation coefficient values, resulting from statistical interpretation using the CORREL software program are presented in table 2.

<table>
<thead>
<tr>
<th>CORRELATION COEFFICIENT</th>
<th>R-M</th>
<th>M-O-T</th>
<th>NE%</th>
<th>SCC</th>
<th>BE</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-M</td>
<td>X</td>
<td>0.32</td>
<td>0.21</td>
<td>0.25</td>
<td>0.40</td>
</tr>
<tr>
<td>M-O-T</td>
<td>0.32</td>
<td>X</td>
<td>0.63</td>
<td>0.65</td>
<td>0.58</td>
</tr>
<tr>
<td>NE%</td>
<td>0.21</td>
<td>0.63</td>
<td>X</td>
<td>0.80</td>
<td>0.78</td>
</tr>
<tr>
<td>SCC</td>
<td>0.25</td>
<td>0.65</td>
<td>0.80</td>
<td>X</td>
<td>0.76</td>
</tr>
<tr>
<td>BE</td>
<td>0.40</td>
<td>0.58</td>
<td>0.78</td>
<td>0.76</td>
<td>X</td>
</tr>
</tbody>
</table>

*have been take in consideration all the absolute values read for all investigated udder quarters

Table 2

The correlation coefficient between the diagnostic tests for mastitis used in our investigations*
Setting certain limits of positive and negative probability of identification of mastitis in each test, we excluded from the statistically calculation the uncertain milk samples, which were named: post control or re-control cases.

Without these re-control cases, the correlation coefficient values have changed significantly for some tests and insignificant for others, according to table nr. 3.

### Table 3

The coefficient of correlation between the diagnostic tests for mastitis used in our investigations, after the post control cases elimination

<table>
<thead>
<tr>
<th>CORRELATION COEFFICIENT</th>
<th>R-M</th>
<th>M-O-T</th>
<th>NE %</th>
<th>SCC</th>
<th>EB</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-M</td>
<td>X</td>
<td>0.46</td>
<td>0.46</td>
<td>0.39</td>
<td>0.39</td>
</tr>
<tr>
<td>M-O-T</td>
<td>0.46</td>
<td>X</td>
<td>0.64</td>
<td>0.59</td>
<td>0.40</td>
</tr>
<tr>
<td>NE %</td>
<td>0.46</td>
<td>0.64</td>
<td>X</td>
<td>0.85</td>
<td>0.76</td>
</tr>
<tr>
<td>SCC</td>
<td>0.39</td>
<td>0.59</td>
<td>0.85</td>
<td>X</td>
<td>0.70</td>
</tr>
<tr>
<td>EB</td>
<td>0.39</td>
<td>0.40</td>
<td>0.76</td>
<td>0.70</td>
<td>X</td>
</tr>
</tbody>
</table>

The basic rules for the interpretation of the correlation coefficient is as follows:
- a correlation coefficient of -0.25 to 0.25 means a low or zero correlation;
- a correlation coefficient of 0.25 to 0.50 (or from -0.25 to -0.50) means a certain degree of association;
- a correlation coefficient of 0.5 to 0.75 (or from -0.5 to -0.75) means a moderate to good correlation;
- a correlation coefficient greater than 0.75 (or less than -0.75) means a very good association or correlation.

It is important to notice that all tests which were used reported a positive correlation. This observation, which is in accordance with data from the literature, means that as the installation of an infectious process in the mammary gland, the studied parameter values are increasing.

Careful analysis of the table 3 revealed that, taking into account only certain results, after a single reading, the results of all the readings, all tests denotes a degree of association as "acceptable", most readings hovering in the range of 0.5-0.75, which implies a "moderate to good correlation".

This implies that, to an extent greater than or less than, all tests used have provided results confirmed in varying degrees by other tests, proving useful under certain circumstances.
However, outstanding results have provided those tests which showed a very good combination, with a higher correlation coefficient than 63% (0.63).

In this sense, the most accurate results provided the percentage of neutrophils, which was correlated to the highest degree (0.76 and 0.85) with SCC and bacteriological exam.

However, the results must be appreciated in the context of taking into account other factors, sometimes with decisive value, such as: price/sample, the workload and time of execution, the possibility of the testing on individual collective samples.

In this way, all five tests have proven their validity (given by the positive correlation at least acceptable), but in other circumstances, namely the different purposes, such as:
- some are suitable to apply on individual samples, from each cow (R-Mastitest and Mast-O-Test);
- others can be preferably to apply on samples from bulk tank (SCC);
- others can be carried out to confirm the results obtained by the first two categories (the percentage of neutrophils and bacteriological exam).

Based on all the results obtained and taking into account the factors mentioned above, we believe that we are entitled to recommend for wide practice pairing of two tests for performance testing of cows, one of which is to reveal the changes in physico-chemical properties of milk (Mast-O-Test) with a test to reveal cytological changes (percentage of neutrophils, and bacteriological exam). R-Mastitest can be classified into either of two categories.

**Conclusions**

As a result of its own investigations relating to the values of indirect or direct tests for diagnosis of subclinical mastitis of dairy cows, were dislodged the following conclusions:

Research has shown that, after the first exam (not taking into account the post control cases), all five tests provide results that correlate positively, within the limits of coefficients included between 0.33 – 0.85, but that the best correlation was observed between:
- the percentage of neutrophils – somatic cells count (0.85);
- the percentage of neutrophils – bacteriological exam (0.76);
- Mast-O-Test – SCC – the bacteriological exam (0.70).

It appears that two of these tests are based on evidence of cytological changes (percentage of neutrophils and the determination of the total number of somatic cells) and one test is based on the study of physical-chemical changes in milk (Mast-O-Test).

Taking into account the results of the reporting data, the bacteriological exam, neutrophil percentage and total number of somatic cells, it has been revealed that the most accurate and reliable results regarding subclinical mastitis of
dairy cows diagnosis could be obtained by examining samples of milk through two tests simultaneously, one of which based on cytological changes and another on physical-chemical transformations. The best combinations that would impose in this regard would be: Mast-O-Test with neutrophil percentage or Mast-O-Test with bacteriological exam.

Automatic determination of the number of somatic cells is an expensive test and is recommend only to confirm the results of other tests or to indicate the milk quality.

References

RESEARCH ON PRESENCE OF VIRUSES IN BROILERS

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Summary

In intensive poultry broilers infectious and contagious disease progresses more viral etiology. In the epidemiological situation of farms, the disease may evolve clinical or subclinical, is usually complicated by secondary bacterial infections. Also, the viruses that cause these diseases induced in broilers body, immunosuppressive conditions that adversely affect the immune response after vaccination against other diseases.

Given those issues, research aimed presence of viruses in 10 broiler farms, samples were taken from cadavers aged between 15 and 35 days.

The viruses were detected by polymerase chain reaction for DNA viruses by polymerase chain reaction with reverse transcriptase for RNA viruses.

These two tests of molecular biology, viruses were detected following: avian reovirus (in 10 farms), infectious bronchitis virus (in 6 farms), infectious bursal disease virus (in 2 farms), chicken parvovirus (in 4 farms), nephritis virus infectious (in 2 farms), astrovirusul chicken (on a farm). Not identified viruses: rotavirus avian infectious, anemia virus to chickens and chickens proventricular necrosis virus.

Key words: poultry, viral diseases, PCR, RT PCR

In broilers from intensive evolving infectious diseases endemic ribo or adenoviruses. These diseases can develop clinical or subclinical, being, frequently complicated by secondary bacterial infections. These viruses are accompanied by immunosuppressive conditions that may influence the immune response after vaccination or suggestive may favor the emergence of other infectious diseases (5, 6, 7).

The emergence and evolution of these diseases in broiler flocks produce significant economic damage mortality through treatment costs through increased the specific consumption and reduced weight gains (5, 6, 7).

Research has followed the presence of viruses in flocks of broilers, in 10 farms at different ages.

Materials and methods

The research was conducted in 10 farms of broilers in the west of the country throughout the year. Samples were taken from cadavers aged 14-35 days and were represented by fragments of lungs, proventriculus, small intestine, spleen and Bursa Fabricius.
For detection of viral DNA was used for polymerase chain reaction (PCR) and for detection of RNA viruses were used for polymerase chain reaction with reverse transcriptase (RT-PCR) (2, 4).

Viral nucleic acids were isolated using the kit QLAamp viral RNA Mini Kit (Qiagen, Germany) and the High Pure Viral Nucleic Acid Kit (Roche, Switzerland), and virus detection was performed using primers 9, in which the characters are shown in table 1.

### Table 1

<table>
<thead>
<tr>
<th>Farms</th>
<th>Age (days)</th>
<th>IBV</th>
<th>ARV</th>
<th>AvRV</th>
<th>IBDV</th>
<th>CAV</th>
<th>ChPV</th>
<th>ANV</th>
<th>CAstV</th>
<th>CPNV</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>14</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>A</td>
<td>21</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>A</td>
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<tr>
<td>A</td>
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<td>+</td>
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<td>+</td>
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<td>B</td>
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<td>+</td>
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<td>-</td>
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<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C</td>
<td>14</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
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</tr>
<tr>
<td>E</td>
<td>21</td>
<td>-</td>
<td>+</td>
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<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
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<td>21</td>
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<td>-</td>
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<tr>
<td>G</td>
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<td>+</td>
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<td>-</td>
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<td>+</td>
<td>+</td>
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<td>-</td>
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<td>-</td>
<td>-</td>
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<tr>
<td>J</td>
<td>35</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

In the case of DNA viruses, temperature profile assumed distortion, amplification and DNA extension; these three steps are carried out in 35 successive cycles.

In the case of RNA viruses, the temperature profile was the same phase and the same number of cycles, but the reverse transcription was carried out at 50° for 30 minutes.

Visualization of amplicons was performed by agarose gel electrophoresis and the resulting bands were visualized by UV TFX 35M device 312 nm UV transilluminator (Life Technologies, UK). Subsequently, amplicons were photographed and analyzed using software appliance and software Kodak Digital Science ID software (Kodak, Japan).

### Results and discussions

Using PCR and RT-PCR tests in broiler flocks, of the 10 farms investigated were found 6 viruses, and 3 viruses was not detected, the results are shown in Table 2.
### Table 2

<table>
<thead>
<tr>
<th>Virose</th>
<th>RNA/DNA</th>
<th>Gene</th>
<th>Primer name and the sequence (5’-3’)</th>
<th>Size Amplicon (pb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARV</td>
<td>ARN</td>
<td>S4</td>
<td>S4-F13:GTG CGTGTTGGAGTTTCCCG S4-R1133:TACGCCATCCTAGCTGGA S4-ORF1:AGAGAGGCCTTGGCTGCTTTG S4-ORF1R:CTCTAACCCTGGCATATCTTCT</td>
<td>1120</td>
</tr>
<tr>
<td>ANV</td>
<td>ARN</td>
<td>GP1</td>
<td>ANV-ORF1F:AGAGAGGCCTTGGCTGCTTTG ANV-ORF1R:CTCTAACCCTGGCATATCTTCT</td>
<td>608</td>
</tr>
<tr>
<td>CaSTV</td>
<td>ARN</td>
<td>ORF 1b</td>
<td>ANVpol1F:GTYGGCCGCGYCTYTTGAYAC ANVpol1R:CRT TTGCCCKRTARTCTTTRT</td>
<td>373</td>
</tr>
<tr>
<td>IBV</td>
<td>ARN</td>
<td>N</td>
<td>IBV-In1F:GTGATGACAAAGATGAATGAGGA IBV-In2R:rCAGATGAGGTCATTGCTTATC</td>
<td>402</td>
</tr>
<tr>
<td>AvRV</td>
<td>ARN</td>
<td>NSP4</td>
<td>NSP4-F30:GTGCGGAAAGATGAGGAAC NSP4-R660:GTTGGGGTACCAGGGATTAA</td>
<td>630</td>
</tr>
<tr>
<td>IBDV</td>
<td>ARN</td>
<td>VP2</td>
<td>IBDV-VP2F:GCCCAAGAGCTACACCAT IBDV-VP2R:rCCGGATTATGTCTTTGA</td>
<td>743</td>
</tr>
<tr>
<td>CAV</td>
<td>ADN</td>
<td>VP1</td>
<td>CAV-VP1-910:TTGGCACCACCTCAAGCGACT CAV-VP1-1262:rCCGTCCCGAATCAACTCAC</td>
<td>353</td>
</tr>
<tr>
<td>ChPV</td>
<td>ADN</td>
<td>NS1</td>
<td>ChPV-F1:TTGAGATGACAAAGATGAATGAGGA ChPV-R1:GGGCGTAACCATTCAGATA</td>
<td>561</td>
</tr>
<tr>
<td>CPNV</td>
<td>ARN</td>
<td>VP1</td>
<td>CPNV-F1:TTGAGATGACAAAGATGAATGAGGA CPNV-R1:GGGCGTAACCATTGCCAGATA</td>
<td>171</td>
</tr>
</tbody>
</table>

Avian reovirus (ARV) was identified in all samples from the cadavers of different ages in broiler flocks. That this virus had the highest frequency calls for a development malabsorption endemic syndrome and arthritis and tenosynovitis farms investigated phenomenon confirmed by other authors (1, 3, 7).

Infectious bronchitis virus (IBV) had a lower frequency than avian reovirus, were identified in six farms at the age of 14, 21 and 35 days. In farms where the virus has been identified, it has evolved avian infectious bronchitis, suggesting that it may be strains of this virus mutant (2,7).

Chicken parvovirus (ChPV) had a low frequency, being isolated only three farms, chickens aged 14, 28 and 35 days.

Infectious bursitis disease virus (IBDV) was found in only two farms in chickens aged 28 days. Reduced frequency of this virus is the result of Infectious Bursal Disease prevention through systematic vaccination.

Avian nephritis virus (ANV) was found in only two farms at the age of 14 and 28 days is considered a part-time virus in broiler flocks (5, 6, 7).

Chicken astrovirus (CaSTV) was identified only in a broiler farm at the age of 35 days is considered all-time virus (5, 6, 7).

In the samples examined were identified following viruses: Chicken anemia virus (CAV), Avian rotavirus (AsRV) and chicken proventricular necrosis virus (CPNV).
The obtained results proved that the broiler farms investigated were detected 6 viruses that cause diseases endemic evolving infectious and contagious specific intensive poultry farming. These viruses are frequently reported in several countries as a result of their dissemination through trade in poultry material (1, 3, 7).

These results are similar to data in the literature, the study of these viruses and the diseases caused collective attention of several researchers. (3, 5, 6, 7).

Conclusions

By PCR and RT-PCR techniques were detected 6 viruses that cause disease specific infectious and contagious intensive poultry farming.

Identification of avian reovirus in all broiler farms shows a high prevalence of infections caused by this virus.

Chicken astrovirus avian nephritis virus and had a reduced frequency and Chicken anemia virus, Avian rotavirus and chicken proventricular necrosis virus was not identified.

References

THE SEROPREVALENCE OF REOVIRUS INFECTIONS IN BROILER FLOCKS

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Summary

Reovirus infection are infectious diseases and intensive poultry farming affects, mainly, broilers, evolving or as malabsorption syndrome or syndrome as arthritis, tenosynovitis.

The investigations were made in order to determine seroprevalence of these infections in six broiler farms west. Blood samples were taken from chickens aged 21 days (R1) and 37 days (R2). Specific antibodies were detected by ELISA (Enzyme Linked Immunosorbert Assay) kit using FlockChek® Avian reovirus Antibody Test Kit, supplied by IDDEXX Laboratories, Inc.

At the age of 21 days geometric mean titres have different values, limits ranging between 22 and 245 OD. At the age of 37 days, the geometric means of specific antibody titers were higher limits ranging between 648 and 773 OD.

The results obtained demonstrating the existence of seroconversion phenomenon is the result of evolution reovirus infection in broiler farms investigated.

Key words: reovirus infection, broiler, seroconversion phenomenon.

Reovirus infections in poultry are spread throughout the world and are considered the specific infectious diseases intensive poultry farming. In chickens OLSON et al., in 1957, described arthritis and tenosynovitis, and DALTON et al., in 1967, proposed the term tenosynovitis to name these conditions (2, 5, 8).

Later, Kouwenhoven, in 1978, in the Netherlands, described, all in broilers, a clinical form with digestive localization, as the malabsorption syndrome (2, 5, 8). It was later shown that avian reovirus isolated by WALKER et al., is the etiologic agent of avian reovirus two syndromes (3, 4).

Commerce intensive poultry material, after 1990, contributed to the emergence and spread of these infections in our country (1, 5).

The research was made in order to determine the seroprevalence of these infections in six broiler farms in western country.

Materials and methods

For establishing seroprevalence reovirus infections were performed serological tests specific antibodies were detected by ELISA (Enzyme-Linked
Immunosorbent Assay) using Flock Chek Kit Avian Reovirus Antibody Test Kit supplied by IDEXX Laboratories Inc.

Blood samples were taken from the chickens at the age of 21 days (R1) at the age of 35 days (R2), each 25 samples at each sampling.

Results and discussions

The results from serological tests are shown in Table 1. The software kit used for the interpretation of established group's titre minimum, maximum and geometric mean titer of antibody titres and their values were expressed in optical density (OD).

In broilers aged 21 days (R1) antibodies were antireovirus whose minimum titer was between 18 DO and 264 OD and geometric mean values were between 245 DO and 607 D.O.

In broilers, aged 35 days (R2), minimum antibody titers were between 12 antireovirus DO and OD 364 and maximum titers were OD values between 1453 and 3256 DO and geometric mean values were between 89 DO and 773 D.O..

### Table 1

<table>
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</table>


Serological results demonstrate the presence of reovirus infection in broiler farms investigated confirming the suspicion, epidemiologic and clinical established. In these farms has evolved malabsorption syndrome arthritis and tenosynovitis.

Analyzing the minimum and maximum titers and geometric mean that these immunological parameters evolved dynamically, while broiler age.

At the age of 21 days specific antibody titers were low due to progressive exhaustion, they are antibodies yolk, chicken flocks from breeding hens vaccinated. After this age, chickens were immunologically protected and were infected with avian reovirus existing farms. In avian reovirus infection is horizontal and vertical phenomenon shown by many researchers (2, 5, 8).

At 35 days of age-specific antibody titers were higher values indicating such a specific phenomenon of seroconversion postinfectious immune response as
a result of the clinical course of the two syndromes. These results confirm the suspicion of both epidemiological and clinical syndromes of avian reovirosis in chicken flocks investigated.

Postinfectious immune response confirmed the values of maximum titers of specific antibodies have been demonstrated by other researchers as characteristic of these infections (2, 8).

Conclusions

Serological examination conducted by ELISA revealed the presence of antibody titers antireovirus whose values were different by age.

At the age of 21 days in broilers, the values were lower due to the exhaustion of yolk antibody titers after the vaccine.

At the age of 35 days had elevated antibodies, showing an immune response after infection.

The results confirm the evolution of avian reovirosis in broiler flocks from farms controlled.

References

EPIDEMIOLOGICAL SURVEY REGARDING ENZOOTIC BOVINE LEUKOSIS EVOLUTION IN TIMIS COUNTY BETWEEN 2000 AND 2009

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Summary

This study represents an assessment of the evolution, spread and prevalence of enzootic bovine leukosis (EBL) in cattle from Timis County, during 2000-2009, to determine the effectiveness of control methods applied for eradication of the disease. The data about positive cattle were taken from the DSVSA Timis archive and were processed and interpreted. The dynamics of enzootic bovine leukosis in Timis County had a downward trend during 2000-2009, which can be attributed to the effectiveness of the control program, but also to the decrease of the cattle number. Regarding the total number of positive farms from EBL outbreaks, there was a decrease in the first five years, followed by a slight increase until 2009, when it was registered almost the same situation as in 2003. The total number of positive animals peaked in 2002 (456 cattle), in the remainder period being below the average.

Key words: enzootic bovine leukosis, Timis County, prevalence

Enzootic bovine leukosis (EBL) is an infectious disease caused by bovine leukosis virus (BLV), which naturally affects cattle and buffalos. Cattle can be infected at any age, including the embryonic stage. Most infections are subclinical, but a significant proportion of cattle over three years (approximately 30%) develop persistent lymphocytosis and a smaller number lymphosarcomas (tumors) in various internal organs. Clinical signs, if are present, depends on the affected organs. Almost all cattle with lymphosarcomas die, either suddenly or in a few weeks or months after apparition of clinical signs (12).

The disease has been described in countries on all continents, respectively: North America (USA and Canada), where rates of infection have been reported up to 50% for dairy herds, South America (Brazil, Chile, Uruguay, Ecuador, Colombia, Venezuela, Peru, Mexico, Costa Rica and Panama), Australia, Asia (Japan and Taiwan), Africa (South African Republic, Guinea, Malawi, and Zimbabwe), the incidence varying according to interest for control and eradication (3, 4, 5, 8, 10).
In Europe, most outbreaks were recorded in the Baltic Sea countries, followed by Belgium, the Czech Republic, Slovakia, Hungary, Romania, Bulgaria, Albania, Netherlands, Switzerland, Italy, England, Turkey, Yugoslavia, France, Portugal and Israel, where the disease has been attributed to the importation of cattle from countries recognized as "source of leukosis" (1, 2, 7, 11).

The eradication program of enzootic bovine leucosis, by slaughtering the infected animals, is applied especially in EU countries. Romanian state, as a member of this community rallied to rigorous measures against ELB by the implementation of the Program of surveillance, prevention, control and eradication of animal diseases, of those transmissible from animals to humans, animal welfare and environmental identification and registration cattle, pigs, sheep and goats of ANSVSA.

The purpose of this study was to assess the evolution, spread and prevalence of EBL in cattle from Timis County, to determine the effectiveness of control methods applied for eradication of the disease.

Materials and methods

The data necessary for the study were taken from the DSVSA Timis archive, the situation of EBL(+) animals during 2000-2009 being extracted. From the existing DSVSA files, a database was conceived, and the data were processed and interpreted using Excel software (Office 2007). The processed data were from 367 cattle farms, of which three were private farms and 364 households, with one or more animals.

Results and discussions

The number of EBL(+) farms at the beginning of outbreak was variable during 2000-2009: the lowest (one infected household) being reported in 2004, and the highest (164 infected farms) in 2000, and the average number EBL(+) farms was 46 (fig. 1). In the first five years, there has been a significant decrease in the number of positive farms, succeeded by a slight increase up to 2007, as in 2009 to return about the situation in 2003 (fig. 1).

In the same period, the number of farms infected with BLV during the last month of each year had values that ranged from zero in 2000, 2004, 2005, 2006, and 14 in 2003 or 13 in 2009. The period average was four newly infected farms during the last month of each assessed year.

Regarding the evolution of the total number of positive farms in ELB outbreaks, we found values between two (in 2006 and 2007) and 280 (in 2002). Thus, there was a decrease in the total number of positive farms from 2002, until 2003, under the period average (78 positive farms), remaining below this value until 2009, when there were registered 37 positive farms (fig. 2).
Fig. 1. EBL evolution trends related to the number of positive farms between 2000 and 2009

Fig. 2. Total number of positive farms from the beginning of EBL outbreaks between 2000 and 2009 period

Number of farms infected with BLV remained at the end of each year, ranged from zero in 2004 to 35 in 2009, with values above period average (8.5 farms) in 2002 and 2003, and below the average in 2000, 2005, 2006 and 2007 (fig. 3).

The number of cattle infected with BLV, at the beginning of outbreak, was variable: the lowest was observed in 2004 (three sick cattle) and the highest in 2002 (173 cattle), and the period average was 63. There was a significant decline since 2003, which restrain the number of BLV(+) cattle below average.

Data recorded during the survey revealed that the number of newly infected cattle during the last month of each year ranged from zero in 2004-2005 and 25 in 2002. Average value for the period was 8 newly diseased cattle, surpassed in 2003 and 2009, the remaining values being below it.
Fig. 3. Dynamic of farms remain infected with BLV at the end of each year

From 2003 to 2009, the total number of BLV(+) cattle was below the average of 116 animals (obtained for the entire period 2000-2009), ranging from 11 in 2004 and a maximum of 456 animals in 2002 (fig. 4).

Fig. 4. Total number of BLV(+) cattle from the beginning of EBL outbreaks

Analyzing the data on the number of remaining cattle BLV(+) at the end of each year (animals that were not slaughtered) we found that control measures have been effectively implemented only in 2000, 2004 and 2006 when all positive cattle were slaughtered. The ineffective measures were recorded in 2007 and 2009, when 21 and 38 BLV(+) cattle remained at the end of the year, far exceeding the average of the period, which were 9.5.

Regarding the number of dead cattle because of EBL in 2000-2009, it was found that in the years 2002, 2004 and 2009 there were four, one, and two cases, for the rest of the period values being null.
Most positive cattle were slaughtered in 2002 (446 cattle), and 2009 was associated with the most ineffective control measures, only seven cattle being slaughtered (fig. 5).

EBL prevalence in cattle from Timis County during 2006-2009 was 0.36%, being identified 545 positive cattle from 121,214 assessed cattle and 150,972 total cattle (Table 1).

It can be seen that, in general, the number of positive animals gradually decreased, aspect can be attributed to the effectiveness of the control program, but also due to the decrease of livestock.

This descendent trend is seen also in other Counties. Thus Simion (9) reported a reduced incidence of EBL in Vaslui County, from 0.64% in 2003 to 0.22% in 2008, explaining the positive effect of seek cattle slaughtering and limitation of livestock movement.

Also, in another County in western Romania, Bihor, Pop (6) describes both reducing the number of outbreaks and the animals of BLV(+) during 2000-2005.
To assess the EBL evolution in cattle population of Timis County, health index was also calculated. This index represents the ratio between the number of healthy animals and the total number of animals. Health index of a population is an epidemiological indicator that shows the degree of conditions necessary to preserve the health of a population.

The evolution of this index in the last four years of the period evaluated was variable, the lowest values being recorded in 2006, and for the next three years, its values varied between 82% and 83%.

Conclusions

The dynamics of enzootic bovine leukemia in Timis County had a downward trend during 2000-2009, which can be attributed to the effectiveness of the control program, but also to the decrease of the cattle number.

Regarding the total number of positive farms from EBL outbreaks, there was a decrease in the first five years, followed by a slight increase until 2009, when it was registered almost the same situation as in 2003.

The total number of positive animals peaked in 2002 (456 cattle), in the remainder period being below the average.

Control measures were 100% applied only in 2000 and 2006.

The small number of dead animals because of EBL (only seven cases during the entire period) demonstrates the effectiveness of screening in the early detection of the disease.
References


THE EFFICACY OF AN ALBENDAZOLE BASED PRODUCT IN LAMBS GASTROINTESTINAL NEMATODE PARASITIS

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Summary

For the efficacy testing of the product Dufalben 10% (albendazole) two groups of 10 lambs each, aged 5-7 months were randomly selected. The first group was treated with albendazole orally at a dose of 7.5 mg/kg, while the second group was the untreated control group. From both groups faeces were collected on days 0, 2, 4, 7 and 12. From each sample qualitative coproscopic exams (Willis) and quantitative exams (McMaster) as well as coprocultures were performed. In the coproscopic and larvoscopic exams parasitism with Teladorsagia circumcincta, Trichostrongylus colubriformis and Oesophagostomum venulosum was identified. Efficacy of the albendazole treatment was 100%, which proves the absence of chemoresistance to this molecule.

Key words: gastrointestinal nematode, lambs, albendazol, efficacy

Introduction of the benzimidazolic anthelmintics in the treatment of gastrointestinal nematodes was considered a major breakthrough in veterinary practice. Rational use of these compounds may improve the clinical efficacy and the avoidance of any form of resistance. Pharmacokinetic behavior of albendazole was studied in several species of animals: sheep, cattle, horses, donkeys, dogs, pigs, goats, buffalo and human beings (2, 3, 6, 7, 11).

This study aimed to determine the therapeutic efficacy of a product (Dufalben 10%) based on albendazole in natural gastrointestinal nematodes infestations and consequently, possible occurrence of chemoresistance.

Materials and methods

The study was conducted on two groups of Turcana breed lambs, aged between 5 and 7 months, from a private herd in Arad County. The lambs were born from sheep that had a history of gastrointestinal nematode parasitism. In the experiment we used a flock of lambs that had no antiparasitic treatments so far.

Two groups of animals naturally infected with gastrointestinal nematodes were randomly selected: Group I consisted of 10 lambs treated with Dufalben 10%
at a dose of 7.5 mg (albendazole) per kg body weight and Group II consisted also of 10 lambs, represented the control untreated group.

Samples of faeces were collected directly from the rectum and subjected to qualitative and quantitative coproscopic examinations (Willis and McMaster methods). Collection of samples was performed on days 0, 2, 4, 7 and 12 post-treatment and the number of eggs per gram of faeces (EPG) (Mc Master method) was determined. The EPG was calculated using the formula: 
$$\text{EPG} = \frac{N \times 100}{2},$$
where "n" is the number of eggs found in both chambers of the McMaster slide (4).

To identify the species of gastrointestinal nematodes in the investigated lambs, coprocultures were performed, on days 0 and 12, to obtain infesting larvae (L3). These were analyzed for the following characteristics: total length, esophagus length, tail length, length from anus to tail sheath, shape and number of intestinal cells. Species determining was based on identification keys from Dikmans and Andrews (1933) (8).

Methods of calculating the effectiveness of the anthelmintics

The drug effectiveness was assessed by two relations recommended by WAAVP: Presidente and Borgsteede, as follows (12).

For test group day 0 (T1) and day 12 (T2), and for control group day 0 (C1) and day 12 (C2), the following relationships were used:

- Presidente relation (%): \(1 - \frac{[T2/T1 \times C1/C2]}{x 100},\)
- Borgsteede relation (%): \((1 - \frac{T2/T1} \times \text{Global Media subjects on day 0/media control group subjects on day 12}) \times 100,\)

where \(T, T2\) and \(C, C2\) is the number of eggs found in both chambers of the McMaster slide.

Results and discussions

Initial coproscopic investigations (Willis method and cultured larvae examination revealed the following species of nematodes: *Trichostrongylus colubriformis, Teladorsagia circumcinta* and *Oesophagostomum venulosum*, respectively. The same nematode species were found at the end of the experiment, but only in the control group.

EPG's values for the two groups, during the experiment (days 0-12), are shown in Table 1. On day 0, in the treated group, mean EPG's was 1095, with the minimum of 850 and maximum of 1450. After analyzing synoptic undergone of Dufalben treated group, a slight decrease of EPG’s value up to 905 since the second day of therapy, has been observed. From the fourth day of treatment decreased value of EPG's was almost half the value of second day EPG's. In days 7 and 12 EPG was negative in group I and, under the two calculation relationships (Boorgsteede and Presidente), the efficacy of Dufalben was being 100%.

In the control group (untreated) on day 0, we obtained a mean EPG's of 1120, with an EPG minimum of 850 and maximum of 1350. On the second day of the experiment could see a EPG with a mean of 1050 with a minimum of 850 and a
maximum of 1250 could be seen and on the fourth day, the average EPG's was identical. On day 7 and 12, the average EPG's was 1020 and 1300, respectively.

Table 1

<table>
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<tr>
<th>ALBENDAZOL TREATED GROUP</th>
<th>DAY 0</th>
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<th>DAY 4</th>
<th>DAY 7</th>
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<table>
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<th>UNTREATED CONTROL GROUP</th>
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<th>DAY 7</th>
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<td>Maximum</td>
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<td>118,2685</td>
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If in the treated group statistical differences between days 0 and 12 are highly significant (p ≤ 0.001), in the control group the differences are insignificant (p ≤ 0.85) (t test).

In a similar study conducted by Guedes da Cruz et al. (2010) in 34 sheep farms in north-western state of Rio de Janeiro, Brazil, albendazole efficacy was 100%, compared with other anthelmintics tested. In comparison with the results obtained by these authors and by our study, in the Brazilian state of Sao Paulo, Almeida et al. (2010), after testing albendazole, found only 19% efficacy in parasitism with *Trichostrongylus colubriformis* and *Haemonchus contortus* (1, 9).
Similarly other authors reported cases of chemoresistance to albendazole. Thus, in France, Palcy et al. (2008) presented the first case of resistance of gastrointestinal nematodes (Trichostrongylus colubriformis) to benzimidazoles, in sheep. In Italy, Cringoli et al. (2007) identified resistance of the trichostrongyles (T. colubriformis) to benzimidazole compounds in goat farms (5, 10).

Conclusions

In Arad County, in lambs, natural infection with Teladorsagia circumcincta, Trichostrongylus colubriformis and Oesophagostomum venulosum was identified. In the natural infection with gastrointestinal nematodes in lambs, the average efficacy of DUFALBEN, based on albendazole, calculated in lambs aged between 5 and 7 months, was 100%. This shows lack of chemoresistance phenomenon to this product for species: Teladorsagia circumcincta, Trichostrongylus colubriformis and Oesophagostomum venulosum.

References

EPIDEMIOLOGICAL STUDY ON HONEY BEE NOSEMOSIS IN ARAD COUNTY

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Summary

Epidemiological studies of honey bees in Arad County were performed on a total number of 16 apiaries processing 292 samples. Each sample consisted of about 60 honey bees. Nosema diagnosis was achieved through a qualitative method. In a total of 52% of the samples Nosema spp. spores were identified. Out of Nosema spp. positive samples, 54% were from asymptomatic colonies, 30% from bee colonies dying in the latter part of winter, 9% from depopulated colonies, 3% from disappeared colonies and 4% were colonies with diarrhea, respectively. Diversity and severity of clinical signs can guide us to the diagnosis of Type C Nosema, with acute evolution and death before clinical expression.

Key words: Nosema spp., epidemiology, Arad County

Arad County is located in the west of Romania, in Western Plain, on the banks of Mures River, in an area of about 7654 square kilometers. To the north it borders Bihor County, to the east Hunedoara and Alba Counties, Timis County to the south and to the west is the Hungarian border. From east to the west, the landscape of Arad County has different levels, including all forms of terrain: plains, hills and low heights mountains, separated by depressions. The climate of Arad County is temperate continental with submediteranean influence in south. Winters are mild and summers are hot. Annual average rainfall is between 566 mm in the plains and 1,200 mm at altitudes exceeding 900 m (Zarand, Codru Moma and Bihar Mountains) (10, 11). In this climate, growing conditions are favorable to bees. But for the evolution of bee diseases, some faults may be involved in the technology and some favorable conditions. In this context lies and evolution of Nosema.

Nosema studies show that the direct effect of the parasite on the bee colony is linked to the changing of the activity and longevity in working bees and queen (3). The infection in working bees delay or inhibit the development of hypopharyngeal salivary glands and thus compromise the young larvae feeding. Part of eggs produced by the queen infected with Nosema cannot achieve the pupa stage, while artificially infected pupae are resistant to infection. Honeybee population starts to decrease with the spread of Nosema infection. Due to changes in physiological status, infected bees become behavioral older than healthy bees of
the same age (6), starting their picking activity at earlier ages, thereby reducing its lifetime (3). It is known that clinical nosemosis has seasonal incidence with the highest number of cases in the second half of winter and totally isolated cases that may develop chronic throughout the season, depending on environmental factors associated with moisture and low temperature (2, 7, 8). Clinical cases of mortality, diarrhea, depopulation, extinction associated with *Nosema* tend to spread throughout the year.

In this study we aimed to determine the prevalence of nosemosis in Arad County, and to correlate the infection with certain repercussions on bee families and a classification of existing symptomatology.

**Materials and methods**

The study was conducted during 2009 -2011, a total of 16 apiaries in eight localities from Arad County: six apiaries from Arad, four from Curtici and one apiary from each of the following localities: Dorobanți, Macea, Iratosu, Șofronea, Ghioroc and Hălmagiu. Totally 292 samples of bees were collected. Most of the live adult bee samples were collected from the second part of the winter and early spring, and a small part of the samples throughout the year. The first period of the year was chosen for the sampling because, due to predisposing factors, it is the maximum pressure period for *Nosema* infection. From each colony about 60 bees were collected for diagnosis with a value of 95% when 5% of the bees are infected.

In each apiary examined data were recorded on: harvest period, number of samples, apiary location, wintering conditions, mortality, morbidity, depopulation, disappearing and symptoms of diarrhea. Bee samples were examined using qualitative diagnostic method for *Nosema* in bees. The method consists in isolating the abdomens of bees and grinding them with one ml of distilled water for each stomach until a homogeneous suspension is achieved. The mixture is then filtered through a fine sieve. A drop of the suspension obtained is examined between slides at optical microscope under the magnification 40X. *Nosema* spores appear as oval corpuscles, ranging in size from 4 to 6 microns, refringent and with increased membrane well evidenced. Negative result does not exclude infection with *Nosema* but in these situations the spores are at unidentifiable levels using the applied method (12).

**Results and discussions**

Laboratory examination revealed that 167 (52%) of the 292 samples collected from apiaries located in Arad County were diagnosed as positive for *Nosema* spp. (Table 1).
Table 1

<table>
<thead>
<tr>
<th>Apiary Location</th>
<th>Total number of sample s</th>
<th>Positive samples</th>
<th>Specifications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nr. %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arad</td>
<td>5</td>
<td>3</td>
<td>60 Two death colonies</td>
</tr>
<tr>
<td>Sânicolaul mic</td>
<td>6</td>
<td>3</td>
<td>50 Two depopulated colonies</td>
</tr>
<tr>
<td>Bujac district</td>
<td>9</td>
<td>7</td>
<td>78 Two depopulated colonies</td>
</tr>
<tr>
<td>Ceala Forest</td>
<td>7</td>
<td>7</td>
<td>100 Asymptomatic</td>
</tr>
<tr>
<td>Curtici</td>
<td>14</td>
<td>10</td>
<td>71 Asymptomatic</td>
</tr>
<tr>
<td>Curtici</td>
<td>14</td>
<td>5</td>
<td>36 Asymptomatic</td>
</tr>
<tr>
<td>Dorobanţi</td>
<td>12</td>
<td>7</td>
<td>58 Three death colonies</td>
</tr>
<tr>
<td>Macea</td>
<td>8</td>
<td>5</td>
<td>62 Asymptomatic</td>
</tr>
<tr>
<td>Ghioroc</td>
<td>8</td>
<td>6</td>
<td>75 Asymptomatic</td>
</tr>
<tr>
<td>Curtici</td>
<td>14</td>
<td>4</td>
<td>29 One death colony</td>
</tr>
<tr>
<td>Bujac district</td>
<td>10</td>
<td>2</td>
<td>20 Asymptomatic</td>
</tr>
<tr>
<td>Sânicolaul mic</td>
<td>26</td>
<td>6</td>
<td>23 Three colonies with diarrhea, one death colony</td>
</tr>
<tr>
<td>Curtici</td>
<td>22</td>
<td>5</td>
<td>23 Four colonies with diarrhea</td>
</tr>
<tr>
<td>Şofronea</td>
<td>100</td>
<td>73</td>
<td>73 35 death colonies; five disappeared colonies</td>
</tr>
<tr>
<td>Hâlmagiu</td>
<td>7</td>
<td>5</td>
<td>71 Two death colonies</td>
</tr>
<tr>
<td>Iraţoşu</td>
<td>30</td>
<td>19</td>
<td>63 Six death colonies and 11 depopulated colonies</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>292</strong></td>
<td><strong>167</strong></td>
<td><strong>52</strong> 50 death colonies (30 %), Five disappeared colonies (3 %), 15 depopulated colonies (9 %), seven colonies with diarrhea (4 %), 90 asymptomatic colonies (54 %)</td>
</tr>
</tbody>
</table>

Of the total sample diagnosed with Nosema, 54% were asymptomatic colonies, most of them observed during the warm season.

Dead colonies during winter and autumn accounted 30% of Nosema positive diagnosis. In these colonies, at remote visual inspection, it was noted that on the hive mirror plate and fly plate, a large number of dead bees were present. At bee colony examination after hive opening, the deaths were confirmed. In most cases strong colonies entered wintering and died in more than 90%. Bees had fallen off the combs in which partially capped food reserves can be seen. Some of the honey reserves were inadequate, were uncapped, fermented and moldy, which made possible the acute clinically manifested Nosema diarrhea and mortality. In these colonies, in faeces, a large number of Nosema spores were identified.

In late winter and early spring, 9% of bee colonies experienced depopulation syndrome. These colonies were not introduced in winter strong so, after the declarations of beekeepers and the number of frames (8-10) in the spring
only one or at most two intervals with bees have been identified. Most of these colonies had also symptoms of diarrhea.

During summer, bee losses are partly compensated by new young bees, but this loss is reflected on lowering production due to lack of bees foraging and decrease of laying capacity in the infected queen.

Symptoms of diarrhea were present in 4% of bee colonies examined and have always been associated with *Nosema*. Colonies of bees with symptoms of diarrhea were recorded only in the second part of the winter.

The remaining 3% of the positive samples were from colonies missing for more than 90%. Depopulated and disappeared colonies were found in spring and autumn.

Dead colonies diagnosed with *Nosema* were found in most cases in the second month of the year and less in the third month. In only one case *Nosema* was found in bee colonies dying in the autumn. Death of bees in the second part of winter is often caused by *Nosema* fact proved also in this study. Mortality was often associated with *Nosema* diagnosis but not always clinically manifested, because only a small proportion of dead colonies had also symptoms of diarrhea. These findings can guide us to the diagnosis of type C *Nosema*, in which evolution is acute and bee colony dies before specific symptoms (1, 4, 5,).

Colonies diagnosed with *Nosema* associated with symptoms of diarrhea were recorded only in the latter part of winter in January and February. During this period, *Nosema* may clinically develop due to its close relation with predisposing factors: brood developing, the hive temperature rise, increased activity of the wintering bees, increased consumption of energy, loading of digestive tube with the impossibility of his release because this period does not allow flight. Diarrhea caused by various reasons, are represented primarily by infectious diseases, intoxications (with toxic nectar or pollen) wintering on forest honey or uncapped and fermented honey (derived from completing feedings made in late fall) (2, 9, 12).

**Conclusions**

Nosemosis was diagnosed in Arad County in 52% of the cases.

54% of *Nosema* spp. positive samples were from asymptomatic colonies. 30% of the samples diagnosed with *Nosema* were from colonies that died in the second half of winter, 9% were form depopulated colonies, 3% from missing colonies, respectively 4% were colonies of diarrhea associated with the presence of *Nosema* spores.

The diversity and severity of clinical signs can indicate a type C *Nosema*.
References


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PERFORMANCE AND INFECTION DYNAMICS WITH *Eimeria* SPP. IN BROILERS MEDICATED WITH *Artemisia annua* IN COMPARISON WITH LASALOCID AND KEPT IN FIELD CONDITIONS

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Summary

During this study we followed performance and dynamic of infection with *Eimeria* spp. in chicken-broilers reared in field condition and medicated with *Artemisia annua* as natural coccidiostatic in comparison with lasalocid. For this purpose in a flock from a farm were designed three groups of 100 randomly selected broilers of mixed sex each. The chicks were treated for all their life-span with *A. annua* dried leaves in-feed (1.5%), essential oil of *A. annua* via drinking water (0.15 ml/l water) and with Tween 70 (0.75 ml/10 l of water). The remaining chicks from the flock were in-feed medicated with lasalocid (125g/tonne of feed). Number of oocysts per gram faeces was monitored every 2 days from 16 to 38 days of age. When chickens were 25 days species of *Eimeria* were determined by PCR. Furthemore, clinical apperence, mortality, lesion score, body weight gain and feed conversion were registred. Three species of *Eimeria* were identified in the flock and groups: *E. acervulina*, *E. tenella* and *E. maxima*. Chikens in-feed medicated with lasalocid or *A. annua* sheded significantly less oocysts (*p*<0.001) than untreated chickens (Tween group), but chickens in-feed medicated with lasalocid sheded significantly less oocysts (*p*<0.03) than chickens treated with *A. annua*. Between *A. annua* groups there wasn't difference statistic significantly (dried leaves = 8,300; essential oil = 9,742). Clinical signs and bloody diarrhoea were registered only in untreated chickens (Tween group). The lesion score at 15 days age of treated chickens was below 0.5, while in Tween group was 1.26; the highest lesion score was noticed in caecum. The mortality percentage was between 2 and 4. Body weight gain and feed conversion ratio were higher in chickens medicated with lasalocid, followed by chicks medicated with essential oil of *A. annua*.

**Key words:** *Artemisia*, artemisinin, *Eimeria*, poultry, field
Coccidiosis remains one of the most costly diseases of the poultry industry. It is caused by apicomplexan parasites belonging to the genus *Eimeria* which develop in the epithelial cells of the gut. In poultry, there are seven recognized species: *E. acervulina*, *E. tenella*, *E. maxima*, *E. necatrix*, *E. mitis*, *E. praecox* and *E. brunette* (41). In broiler chickens frequently are found *E. acervulina*, *E. tenella* and *E. maxima* (18, 21, 32, 37).

Prevention of diseases is based on in-feed prophylaxis with anticoccidial drugs during lifespan of broilers with a withdrawal period before slaughtering, and more rarely with live vaccines (28, 33, 38). Approximately 84% of broilers were prevented with coccidiostats and 12% by vaccination in Europe in 2006 (12). In Romania, prevention of coccidiosis in broiler farms is based exclusively on use of coccidiostats, vaccination being used partially only in layers and breeders (personal observation). Large-scale and long-term use of anticoccidial drugs over 70 years has led to the development of drug-resistance *Eimeria* spp. strains (5, 33), and consecutively to important economic losses caused mainly by poor weight gain and high feed consumption (19, 23). In the world, the economic losses due to subclinical coccidiosis are estimated at more than 3 billion US$ annually (6).

Taking in account these considerations there is a strongly need for inexpensive alternatives for coccidiosis control. Moreover, in organic farming medication with classical drugs is prohibited or restricted (11). For instance, in case of coccidiosis in some countries coccidiostats are allowed (UK) and in others not (Austria) (26, 43). Live vaccines proved to be efficient, but they are expensive and have adverse effects on early chick growth (40). Nevertheless, vaccines are recommended to restore drug sensitivity of resistant *Eimeria* strains (40).

In the last 15th years, studies on the inhibitory effects of natural products on *Eimeria* and/or on the protective immunity of the host were made (30). Dried leaves of *Artemisia annua* have been used in traditional Chinese medicine for over 2 millennia (25), and sesquiterpene lactone from *A. annua* exhibits high efficacy against several stages of *Plasmodium* (15). Also, there are studies showing that artemisinin limits the replication of *Eimeria* spp. in chickens (2, 4, 31, 42).

In this study, we investigated the anticoccidial effects of *A. annua* (leaf powder and essential oil) in broilers kept in field condition and compared with a classical coccidiostatic, lasalocid.

**Materials and methods**

**Animals and experimental design**

The experiment took place in house four of broiler farm S.C. Oprea Avicom S.R.L. located in Crăiești, Mureș county. In the house was introduced a flock of 19,500 chickens Ross 308 obtained from the hatchery of the same commercial society (Venchi-Sighișoara, Mureș county). Chickens were vaccinated against Newcastle disease three times (aged 9, 10 and 24 days) and against Gumboro disease two times (aged 12 and 18 days). Feed and water were offered *ad libitum.*
The lighting programme consisted in alternative periods of light and dark as follows: 1st and 2nd days, 23 hours light and one hour dark; 3rd to 5th day, 22 hours light and two hours dark; 6th to 21st days, 19 hours light and five hours dark; 22nd day, 20 hours light and four hours dark; and from 23rd day till the slaughtering 21 hours light and three hours dark. The light intensity was of 60-70 lux for all period of rearing.

The flock was divided in four groups: (i) group Aa-p of 100 chickens, treated with A. annua GERM07 as powder in feed (15 kg powder per ton of feed; 1.5%); (ii) group Aa-ee of 100 chickens, treated with A. annua ROMN08 as essential oil in water (0.15 ml per liter of water); (iii) group Tw80 of 100 chickens, treated with Tween 80 in water (0.75 ml per 10 liters of water); and (iv) group Las of remaining chickens, in-feed medicated with lasalocid (Avatec®150 G; Alpharma, Belgium; 125 g per ton of feed). Groups Aa-ee and Tw80 were received feed free of coccidiostatics. All treatments were applied from day-old to 36 days-old.

Artemisia products and HPLC-UV analysis of Artemisia annua

A. annua L. was used as (i) A. annua leaf powder obtained from Anamed A3 cultivar (GERM07) and as (ii) essential oil obtained from a Romanian variety of A. annua (ROMN 08). Leaf powder of GERM07 was obtained by grounding dried leaves with artisanal machinery. The essential oil of ROMN 08 was obtained through a hydro distillation process of plants vegetative parts, blooms and flowers (except long stems and branches) using artisanal machinery.

GERM 07 and ROMN 08 A. annua strains were analysed by HPLC-UV analysis to quantify the concentration of artemisinin, deoxyartemisinin, dihydroartemisinic acid, and artemisinic acid, following the published protocol of Ferreira and Gonzalez (13).

Observations and analytical procedures

Efficacy of Artemisia annua as a natural coccidiostatic was evaluated by (i) clinical signs; (ii) mortality rate; (iii) oocysts shed per gram of faeces (McMaster counting technique); (iv) lesion score; (v) body weight; and (vi) feed conversion ratio. Also, Eimeria species were determined when chickens were 26-days-old.

For oocysts counting, pooled fecal samples were collected every two days from 16 to 38 days-old. At each sampling time, 40 fresh droppings were collected per group and mixed, resulting in approxiamtely 200 g faeces per group. Sodium chloride (sp.gr. 1.20) solution was used as flotation solution. 

Oocysts from samples collected on day 26th of age were isolated, purified and concentrated from faeces with saturated salt solution (36) and sporulated in 2.5% potassium dichromate solution. At the end, polymerase chain reaction was done to identify the species of Eimeria.

Lesion score was evaluated at 15 and 35 days-old for 10 chicks per group each time using a score of 0–4 (24).
Deoxyribonucleic Acid (DNA) extraction

DNA extraction from sporulated oocysts of each group was performed with the commercial kit Isolate Fecal DNA Kit (Bioline; cat. no. BIO-52038) following the manufacturer's instructions using 200µl of sporulated oocysts suspension. The DNA was stored at −20°C till analysis.

Polimerase Chain Reaction (PCR)

*Eimeria* species were identified by single PCR assay using species-specific primers targeting the internal transcribed spacer-1 (ITS-1) as previously described by Schnitzler et al. (34, 35) and Haug et al. (20). Each reaction mixture of 25 µl contained: 2µl DNA sample; 25 pmol of species-specific reverse and forward primers; 12.5 µl MyTaq™ Mix (Bioline, cat. no. BIO-25041); 9µl ultra-pure water (PCR Water, cat. no. BIO-37080, Bioline); and 0.5µl 1% bovine serum albumin. We used Houghton strains of all seven *Eimeria* species that infect chickens obtained from VLA (Veterinary Laboratory Agency Weybridge, UK) as positive controls and distilled water as negative control.

The amplification was performed in *MyGenie™* 96 Gradient Thermal Block (Bioneer). The cycling parameters for the amplification consisted of an initial denaturation at 95°C for 1 min, followed by 35 cycles of denaturation (95°C, 15 s), annealing (58 or 65°C, 15 s) and extension (72°C, 10s), with a final extension at 72°C for 3 min.

The PCR products (8 µl), mixed with loading buffer (2 µl), were separated on a 1.5% agarose gel by electrophoresis, stained with SYBR® Green I Nucleic Acid Gel Stain (Invitrogen). Specific fragments were identified by size using a 100 bp ladder under UV light (BioDoc-It® Imaging Systems, UVP®, VWR International LLC).

Statistical analysis

Normal probability plots were generated based on oocysts per gram of faeces, and lesional score, to verify that data followed an approximate normal distribution (10). Groups were compared using Paired-Samples T Test. Analyses were performed using SPSS software version 19.

Results and discussions

**HPLC-UV analysis of A. annua**

GERM07 *A. annua* leaf powder contained 0.75% artemisinin (Art), 0.18% dihydroartemisinic acid (DHAA), and 0.03% artemisinic acid (AA) (Fig. 1).

ROMN 08 *A. annua* plants vegetative parts, blooms and flowers contained 0.2% artemisinin (Art), and 0.3% artemisinic acid (AA).

Although deoxyartemisinin (DOArt) was also present in both samples, the compound lacks the peroxide bridge found in artemisinin, having a single oxygen atom in the bridge, and believed to have no biological activity (29).
Fig. 1. HPLC-UV (192 nm) chromatogram of a 10-µL injection of GERM 07 (leaf powder). Quantification is in g/100g dry weight.

_Eimeria_ species

Chickens from all groups shed oocysts of _E. acervulina_, _E. tenella_ and _E. maxima_ (Fig. 2).

Fig. 2. _E. acervulina_ 145 pb (A); _E. tenella_ 278 pb (B); _E. maxima_ 205 pb (C). Lines (L)1,7, and 13 represent the standard molecular weight of 100 bp, and lines 2, 8 and 14 positive controls (Houghton strains). Group Las: L3, L9, L15; Group Tw80: L4, L10, L16; Group Aa-p: L5, L11, L17; Group Aa-eo: L6, L12, L18.

Worldwide, in broilers are commonly found _E. acervulina_, _E. tenella_, _E. maxima_ and _E. praecox_ (21, 27, 32). During an epidemiological study by PCR in Romanian broiler farms were identified _E. acervulina_, _E. tenella_, _E. maxima_ and _E. praecox_ (16, 17).
Clinical signs and mortality
Clinical signs as weakness, inappetence, polidipsia, and diarrhoea appeared to 12 chickens from Tw80 group at 19 days of age. Three days later, half of chicks presented bloody diarrhoea, and four of them died after another 3 days (Table 1). The mortality rate to the other groups was lower, as follows: 3.3% in Las group; and 2% in A. annua groups (Table 1).

Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Clinical signs</th>
<th>Bloody diarrhoea</th>
<th>Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Las</td>
<td>0</td>
<td>0</td>
<td>3.3%</td>
</tr>
<tr>
<td>Aa-p</td>
<td>0</td>
<td>0</td>
<td>2/100</td>
</tr>
<tr>
<td>Aa-ue</td>
<td>0</td>
<td>0</td>
<td>2/100</td>
</tr>
<tr>
<td>Tw80</td>
<td>12/100</td>
<td>6/100</td>
<td>4/100</td>
</tr>
</tbody>
</table>

Legend: Las – group treated with lasalocid 125 g per ton of feed; Aa-p – group treated with A. annua as powder, 15 kg per ton of feed; Aa-ue – group treated with A. annua as essential oil, 0.15 ml/l of water; Tw80 – group treated with Tween 80, 0.75 ml/10 l of water.

In a previous study after experimental infection with 10,000 oocysts of *E. tenella* and in-feed treatment with 1.5% *A. annua* as leaf powder, 30% of broilers presented clinical signs of coccidiosis, but no mortality (9).

OPG and lesion score
The number of oocysts excreted by the chickens from experimental groups during rearing period is presented in Fig. 3. The OPG was significantly higher (*p*=0.002) in group Tw80 (mean 25,383). Besides, chickens treated with *A. annua* either leaf powder (GERM07 strain) or essential oil (ROMN08 strain) excreted with 67.3% (mean 8,300), and 61.6% (mean 9,742) respectively less oocysts than those treated with Tween 80. Chickens treated with lasalocid sheded the fewest oocysts (mean 750).

We observed the same trend for oocysts production regardless of group; the peak was earlier for Tw80 group (22 days) and later for Las group (26 days); as for Artemisia groups an intermediate peak (24 days) between Tw80 and Las groups was noticed (Fig. 3).

The lesion scores by groups and segments of the intestine (duodenum, jejunum, and caecum) are presented in Table 2, at 15 and 35 days of age. The highest lesion score was registered in Tw80 group (3.8; 2.6), followed by Aa-p (1.6; 1.4) and Aa-ue (1.2; 0.8) groups, and the lowest in Las group (0.6; 1.0). When chickens were 15 days, the percentage of lesion score reduction in *A. annua* groups was about 57.9-68.3, being higher in chickens treated with essential oil. In comparison to chicken treated with lasalocid the reduction was 84.1%. However, at 35 days, the lesion score was below 1, in all experimental groups, less in Tw80 group for caecum (Table 2).
Fig. 3. Effect of *A. annua* as leaf powder (GERM07) (*Aa*-p), and essential oil (ROMN08) (*Aa*-eo) on the fecal oocyst output in field condition in comparison with lasalocid (Las) and tween 80 (Tw80)

Table 2

<table>
<thead>
<tr>
<th>Group</th>
<th>Duodenum</th>
<th>Jejunum</th>
<th>Caecum</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>a</td>
<td>b</td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>Tw80</td>
<td>1.0</td>
<td>0.8</td>
<td>0.8</td>
<td>2.0</td>
</tr>
<tr>
<td>Las</td>
<td>0.3</td>
<td>0.4</td>
<td>0.2</td>
<td>0.4</td>
</tr>
<tr>
<td>Aa-p</td>
<td>0.4</td>
<td>0.8</td>
<td>0.4</td>
<td>0.2</td>
</tr>
<tr>
<td>Aa-ue</td>
<td>0.4</td>
<td>0.6</td>
<td>0.2</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Legend: a – 15 days age; b – 35 days age; Tw80 – group treated with Tween 80, 0.75 ml/10 l of water; Las – group treated with lasalocid 125 g per ton of feed; Aa-p – group treated with *A. annua* as powder, 15 kg per ton of feed; Aa-ue – group treated with *A. annua* as essential oil, 0.15 ml/l of water.

Previously when we used 1,500 and 10,000 oocyst of *E. tenella* (Houghton strain) for experimental infection and 1.5% *A. annua* leaf powder in diet, the oocysts output reduction was higher, between 87.9 and 90.8% and the reduction of lesion score similar, between 55.5 and 56.3% (8, 9).

**Body weight gains (BWG) and feed conversion (FCR)**

Overall, chickens in-feed treated with lasalocid 125 ppm had the best performances: BWG of 62.0 g/day, and FCR of 1.86. The Tw80 group presented the lowest BWG till 41 days, when Aa-p group presented the lowest BWG (Table 3). It is interesting as Aa-p group had among the best BWG till before slaughtering,
and then changed. Even before slaughtering chickens treated with Tw80 had a good BWG, they consumed more feed (2.12) for one kilogram of body weight than the other groups. Chickens treated with A. annua used between 1.96-1.99 kilograms of feed for one kilogram body weight.

Table 3

<table>
<thead>
<tr>
<th>Group</th>
<th>FCR</th>
<th>BWG (7 days)</th>
<th>BWG (14 days)</th>
<th>BWG (21 days)</th>
<th>BWG (28 days)</th>
<th>BWG (35 days)</th>
<th>BWG (41 days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tw80</td>
<td>2.12</td>
<td>17.7</td>
<td>32.3</td>
<td>49.6</td>
<td>49.0</td>
<td>67.9</td>
<td>62.0</td>
</tr>
<tr>
<td>Las</td>
<td>1.96</td>
<td>18.0</td>
<td>33.4</td>
<td>50.0</td>
<td>59.3</td>
<td>65.7</td>
<td>65.0</td>
</tr>
<tr>
<td>Aa-p</td>
<td>1.99</td>
<td>18.6</td>
<td>38.4</td>
<td>44.4</td>
<td>60.1</td>
<td>71.8</td>
<td>52.2</td>
</tr>
<tr>
<td>Aa-ue</td>
<td>1.96</td>
<td>18.0</td>
<td>35.7</td>
<td>57.3</td>
<td>54.0</td>
<td>66.0</td>
<td>63.6</td>
</tr>
</tbody>
</table>

Legend: FCR – feed conversion ratio; BWG – body weight gain; Tw80 – group treated with Tween 80, 0.75 ml/10 l of water; Las – group treated with lasalocid 125 g per ton of feed; Aa-p – group treated with A. annua as powder, 15 kg per ton of feed; Aa-ue – group treated with A. annua as essential oil, 0.15 ml/l of water.

Almeida et al. (3) in medium and low-growing chickens found no significant differences in feed consumption between groups, treated or not with A. annua, even before Eimeria infection chicks treated with A. annua had significantly lower average body weight gains. The authors suggested that the bitter taste of plant had interfered negatively with the broilers’ performance before the build-up of coccidia infections.

The studies made on effect of A. annua against coccidial infection in chickens varied greatly in results. On the plant side, this variation was probably a consequence of artemisinin concentration caused by either different chemotypes or seasonal variation (39), although different methods of drying can also change artemisinin concentration in leaves (14). On the animal side, it was probably a consequence of routes of administration, experimental design (Eimeria species, oocysts dose, etc.), and different susceptibilities of Eimeria species and strains used in the studies. In general, most of them provided a good control against E. tenella infection, but not for E. acervulina and E. maxima (1, 4).

Conclusions

The results obtained by us in chickens kept in field condition suggested that A. annua as leaf powder or essential oil prevents successfully clinical coccidiosis. Moreover, A. annua can be a good candidate for preventing coccidiosis in organic farming.

Acknowledgements

This work was partly supported by UEFISCDI, project number PN II-PCCA Tip 2 110/2012.
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SEROEPIDEMIOLOGY OF *NEOSPORA CANINUM* AND *TOXOPLASMA GONDII* INFECTIONS IN DOGS FROM SOUTHERN ROMANIA

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Summary

*Neospora caninum* and *Toxoplasma gondii* are very closely related protozoans with many common hosts, including dogs. The aim of this study was to evaluate the seroepidemiology of *Neospora* and *Toxoplasma* infections in dogs from southern Romania (Ilfov, Giurgiu, Ialomița, Călărași and Teleorman counties). For this, serum samples from 92 dogs (33 farm dogs and 59 stray dogs) were tested by commercially available indirect immunoenzymatic assays for detecting *Neospora* and *Toxoplasma* specific IgG antibodies. Of the 92 dog tested sera, eight were positive for *Neospora* (8.7%) and 46 were positive for *Toxoplasma* (50.0%). A number of six samples were positive for both *Neospora* and *Toxoplasma* (6.5%). One positive sample for each *Neospora* and *Toxoplasma* were classified as doubtful (1.1%). Prevalence of *Neospora* antibodies was significantly higher in farm dogs (27.3%), compared with stray dogs (5.1%), but difference was not significant when analyzing *Toxoplasma* antibodies, with similar prevalence values in farm and stray dogs (49.2% and 51.5%, respectively). Significant differences were observed between *Neospora* and *Toxoplasma* infections in both farm and stray dogs. The seropositivity for both *Neospora* and *Toxoplasma* increased with age suggesting post-natal exposure, but only for *Toxoplasma* the differences were statistically significant. Prevalence values for *Neospora* and *Toxoplasma* in age categories were as follows: 0% and 16.7% in dogs under 1 year, 12.5% and 54.2% in dogs of 1 to 5 years, 20% and 60% in dogs of 6 to 9 years, and 40% and 100% in dogs of 10 years and over, respectively. No significant differences were observed for the presence of *Neospora* and *Toxoplasma* antibodies between male and female dogs. In conclusion, dogs from southern Romania have been in contact with both protozoans, but *Toxoplasma* antibodies were detected in much higher rates. Farm dogs were more exposed to *Neospora* infection than stray dogs, while both farm and stray dogs were exposed to *Toxoplasma* infection. Altogether, these findings are emphasizing high risks for both, public health and cattle farming industry.

**Key words:** dogs, ELISA, *Neospora caninum*, Romania, *Toxoplasma gondii*.

*Toxoplasma gondii* is known for more than a century, while *Neospora caninum*, a very closely related apicomplexan, was differentiated from the first one for only a quarter of a century (5).

*Neospora* and *Toxoplasma* infections are distributed worldwide and both parasites are capable of infecting various species of domestic and wild animals. *Neospora* is a major cause of abortions and congenital infections in cattle (4) while toxoplasmosis causes abortion and congenital infection especially in sheep and goats and is a major zoonosis (5). Although *Neospora caninum* and *Toxoplasma*
*gondii*, usually, does not produce clinical signs of disease in adult dogs, this infections are epidemiologically important because the dog is the definitive host of *Neospora* (14) and may be considered as a sentinel in the epidemiology of *Toxoplasma* (6).

Diagnosis of both *Toxoplasma* and *Neospora* infections in dogs is based on serological assays, such as IFAT and ELISA (4). *Toxoplasma* infection in dogs has been reported in different countries around the world with seroprevalence rates ranging from 12.8% in Korea (17) to 52% in Brazil (15). Usually, for *Neospora*, the seroprevalence ranges between 0 and 20% in different dog populations without clinical signs of neosporosis (20).

*Neospora* seroprevalence in dogs by IFAT was 20.2% in a recent study from southern Romania (16).

*Toxoplasma* infection in animals has been extensively studied in Romania, especially in the west, north and centre of the country. Recent studies reported a seroprevalence of 23.1% in pigs and 16% in wild boars (18) and 52.8% in goats (11). In Caras-Severin County, Hotea et al. (10) reported high prevalence rates of *Toxoplasma* infection in sheep (61.33%) and cats (77.42%). Different animals species raised in captivity in the Timișoara Zoological Garden were also tested for *Toxoplasma gondii* antibodies and the overall prevalence was 73.07% (3).

However, only limited surveys of *Toxoplasma* infection in dogs have been reported in Romania. Cozma et al. (2) reported a 24.56% seroprevalence of *Toxoplasma* infection in stray dogs from Cluj County, but, to our knowledge, no published data about seroepidemiology of *Toxoplasma* infection in dogs from southern Romania is available.

Therefore, the aim of this study was to evaluate the seroepidemiology of *Neospora* and *Toxoplasma* infections in dogs from southern Romania by indirect ELISA method.

**Materials and methods**

To investigate the exposure of dogs to *Neospora* and *Toxoplasma* infections, the specific antibody response was evaluated using 92 dog serum samples collected in 2011 and 2012 from different rural and urban areas located in southern Romania. Dogs randomly selected for individual sampling were divided in two categories: farm dogs (n=33) originating from different dairy cattle farms located in Ilfov, Giurgiu, Ialomiţa, Călăraşi, Prahova and Telorman counties and stray dogs (n=59) originating from Bucharest and Pantelimon cities.

Dogs were of common breed, females and males, 2 months - 14 years old. None of them showed neurological disorders (paresis, ataxia, and myoclonus) or other clinical symptoms associated to neosporosis or toxoplasmosis, except two stray dogs from Bucharest.

In order to detect the anti-*Neospora* and anti-*Toxoplasma* antibodies of the IgG class, two commercially available multi-species indirect ELISAs were used: ID
Screen® *Neospora caninum* Indirect Multi-Species and ID Screen® *Toxoplasma gondii* Indirect Multi-Species, ID-VET Lab., Montpellier, France).

The two assays were performed following exactly the manufacturer’s instructions. The optical density values were read at 450 nm, using a spectrophotometer. The cutoff of the tests was S/P≥50% (obtained by an equation provided by the manufacturer). Samples with S/P between 40 and 50 were considered doubtful.

Analysis of the data was performed using Fisher’s exact test or Chi-square ($\chi^2$) test by Quantitative Parasitology 3.0 software. Statistical significance was assumed at $p≤0.05$.

**Results and discussions**

Only eight dogs were positive for anti-*Neospora* antibodies (8.7%; CI$_{95\%}$=3.82 – 16.42) while anti-*Toxoplasma* antibodies were found in 46 of the 92 dogs (50.0%; CI$_{95\%}$=39.38 – 60.62). A number of six samples were positive for both anti-*Neospora* and anti-*Toxoplasma* antibodies (6.5%; CI$_{95\%}$= 2.43 – 13.66). One positive sample for each *Neospora* and *Toxoplasma* were classified as doubtful (1.1%; CI$_{95\%}$= 0.02 – 5.91).

The prevalence found for *Neospora* in the present investigation by indirect ELISA, is lower than previously obtained by IFAT (20.2%) in similar studies in the same area (16), possible because different diagnostic tests were used. Antigenic cross-reactivity between *Neospora* and *Toxoplasma* and potential false positive results due to *Toxoplasma* are possible, according to the producer of the IFAT kit used in the study mentioned above (16).

Our seroprevalence rates for *Neospora* were 5.7 times lower than those for *Toxoplasma*. Similar results, with much higher prevalence rates for *Toxoplasma* (45.1% - 52%) than for *Neospora* (8.4% - 9.8%) were reported for canine populations from Brazil (1, 15). Also in Austria, *Toxoplasma* recognized a higher prevalence in dogs (26%) than *Neospora* (3.6%), as reported by Wanha et al. (21).

Co-infections were more common in our study (6.5%) than in other similar studies: 0.72% in Korea (17), 1.7% in Austria (21), and 4.9% in Brazil (1).

In Iran nearly similar prevalence values were reported for *Toxoplasma* (26.8%) and *Neospora* (29%) infections in dogs, with a higher level of co-infections (8.94%), by using indirect ELISA tests (9).

The results obtained in the present investigation were analyzed by living places, sex, and age categories and were summarized in Table 1.
Table 1

Neospora and Toxoplasma seroprevalence in dogs, according with the lifestyle category, sex and age

<table>
<thead>
<tr>
<th>Lifestyle category of dogs</th>
<th>Sex</th>
<th>Age (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Farm</td>
<td>33</td>
<td>59</td>
</tr>
<tr>
<td>Stray</td>
<td>29</td>
<td>63</td>
</tr>
<tr>
<td>Neospora positive (%)</td>
<td>9</td>
<td>(27.3)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>(5.1)</td>
</tr>
<tr>
<td>Statistical difference</td>
<td>p=0.007</td>
<td>p=0.327</td>
</tr>
<tr>
<td>Toxoplasma positive (%)</td>
<td>17</td>
<td>(51.5)</td>
</tr>
<tr>
<td></td>
<td>29</td>
<td>(49.2)</td>
</tr>
<tr>
<td>Statistical difference</td>
<td>p=0.827</td>
<td>p=0.822</td>
</tr>
</tbody>
</table>

Significant differences were observed between Neospora and Toxoplasma infections in both farm (p=0.044) and stray dogs (p=0.0001).

For Neospora infection, rate of seroconversion was significantly higher in farm dogs than in stray dogs (p=0.007). This may be explained by an easier access of farm dogs to infected tissues of bovine origin. Therefore, farm dogs may represent a potential infection risk for cattle in the area, considering their role as definitive host for Neospora. The seroprevalence of Neospora infection in cattle in southern Romania, has been found as 34.9% (7).

Some other researchers reported that dogs living in rural areas, including farm dogs, were significantly more exposed to Neospora infection, than dogs living in urban areas, including stray dogs (19, 21).

Toxoplasma antibodies were detected with similar prevalence rates (p>0.05) in farm (51.5%) and stray (49.2%) dogs, so we can assume that the infectious material (sporulated oocysts or raw meat) could be accessed equally by both dog categories. Neospora and Toxoplasma infections can be horizontally transmitted in dogs by carnivorism (ingestion of tissues infected with tachyzoites or tissue cysts) or by accidental ingestion of sporulated oocysts (5). Lopes et al. (13) identified some risk factors for acquiring Toxoplasma infection in dogs from northeastern Portugal (38% prevalence), such as: eating birds or small mammals, housing exclusively outdoors, home-cooked meals, and eating raw meat or viscera.

Dogs do not have a biological role in the transmission of Toxoplasma. However, in a recent study (6), stray dogs are considered as sentinels in the epidemiology of Toxoplasma because of their eating habits. Furthermore, dogs may represent a risk for public health regarding Toxoplasma infection, because dogs may act as mechanical vectors for Toxoplasma oocysts (8, 12).

Regarding genders, in the present study there were no significant differences in seroprevalence between males and females, for neither Neospora or Toxoplasma infections, in agreement with other surveys (1, 9, 17). This may be
explained by the fact that both male and female dogs are exposed to the same environmental risk factors.

Seroprevalence for both *Neospora* and *Toxoplasma* infections increased with age, but statistically significant differences were observed only for *Toxoplasma* infection ($p<0.05$), reaching a 100% prevalence in dogs aging 10 years or older. Regarding *Neospora* infection, no dog aging less than 1 year presented specific antibodies. These findings emphasize an elevated risk of pathogen contact with increasing age of the dog, and support the horizontal transmission pattern, as well as others previously observed (1, 2, 9, 15).

Concerning the two dogs with neurological disorders, one of them presented a high antibody titer for anti-*Toxoplasma* antibodies ($S/P= 291.91\%$) and was negative for *Neospora* infection. This dog, 14 month old, presented myoclonus and ataxia at the clinical examination, and a history of distemper-like symptoms with one month before (fever, purulent nasal and ocular discharge, dyspnea), but the rapid antigen test for canine distemper virus was negative. These symptoms are consistent with clinical toxoplasmosis (5). The other dog with neurological disorders, two month old, was negative for both *Neospora* and *Toxoplasma* infections.

**Conclusions**

The higher seroprevalence of farm dogs for anti-*Neospora* antibodies obtained here (27.3%) suggests a potential *Neospora* infection source for cattle in the area.

The higher prevalence of anti-*Toxoplasma* antibodies in canine populations from southern Romania (50%) may indicate a high environmental contamination in the area, pointing the need for more epidemiological studies. In addition to its veterinary importance, toxoplasmosis is of major zoonotic concern and dogs can serve as epidemiological indicators for local infections.

The seroprevalence rates increased with age, for both *Neospora* and *Toxoplasma* infections, suggesting predominance of a horizontal transmission pattern in dogs from the studied area.

**Acknowledgements**

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DIAGNOSIS AN OUTBREAK OF PRRS BY PCR ON A FARM IN BRAILA COUNTY

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Summary
Porcine Reproductive and Respiratory Syndrome (PRRS) is an infection contagious disease of viral nature, found in pigs. Actually, the cause of the disease produces economic damage through the breeding and respiratory disorders, as well as by the prevention and control spending.

This study is built on the research on PCR (polymerase chain reaction) in pigs from a professional breeding unit.

Following the investigations carried out for detection of viral RNA, the most positive reactions were obtained at piglets' category; they are the most susceptible to this disease.

Key words: swine, PRRS, RT-PCR.

Polymerase chain reaction (PCR), is one of the techniques that can be used to successfully detect the PRRSV genome in the fluid (2).

PCR is recommended for the samples that are toxic to the cell cultures and for those in which the PRRSV has been inactivated, or is neutralized by antibodies.

In some laboratories, the PCR technique is used almost exclusively for the confirmation of suspected cases of PRRSV.

Polymerase chain reaction is the wave of the future in diagnostics, because it increases sensitivity (it can detect as few as 10 organisms in a sample), specificity, and speed (it can be completed within several hours). Any type of sample can be used with PCR, which identifies pathogen (virus, bacteria, or parasite) and host genes (5).

The genome of PRRSV consists of 8 open reading frames (ORFs), 6 of which are expressed by the formation of subgenomic RNAs.

Polymerase chain reaction (PCR), a sensitive and specific assay for the diagnosis of many infectious diseases, has recently been described for the detection of PRRSV in clinical samples, including boar semen.

One of the critical parameters in the PCR technique is the selection of the primer pairs, because nucleotide mismatch could lead to false negative results (3).
Materials and methods

Holding of growing pigs under study is located in the county of Braila, a complex type location, intercooperativ, aloof from human communities. It is a professional unit with continuous flow, with distinct areas: breeding, maternity, increasing youth and fattening.

In this farm disease began shortly after the actual introduction of newly acquired lots of animals. From the dead bodies brought were collected the organ samples in a total number of 18 and to tissue homogenates were made. Organ homogenate was obtained in tubes Green Ceramic Ball and phosphate buffer pH - 7.2. Homogenization was made in the Magna Lyse. Were taken 200 µl of supernatant from homogenate such prereded, in which ARN was extracted according to the procedure below. With the investigated samples were used and two witnesses (a positive control - vaccine from Intervet and negative control - ultra pure water).

Protocol

- Centrifugation of the samples for 3 minutes at 13.000 rpm at 4°C
- The supernatant is introduced into 1.5 ml Eppendorf tubes then add 70% Ethanol (1 ml water 3 ml absolute ethanol - import).
- Mix by pipetting
- Transfer in minicolumn to the upper lip of the outer tube
- Centrifugation at 13.500 rpm for 15 sec
- Removal of supernatant;
- Buffer RW 1
- Centrifugation at 13.000 rpm for 15 sec
- Removal of supernatant;
- Working Buffer RPE (11 ml RPE conc. + 44 ml ethanol)
- Centrifugation at 13.000 rpm for 15 sec
- Removal of supernatant;
- Working Buffer RPE
- Centrifugation at 13.000 rpm for 3 min
- Insert filter into a 1.5 ml tube Ependorff
- RNase-free water
- Centrifugation at 13.000 rpm for 1 min

Before coupling ARN extracted, prepare the reaction mix, which consists of reagents from the kit "OneStep RT-PCR" (5X reaction buffer, enzyme mix, dNTP's and ultra pure water), hydrated PRRS primers and RNasin.

Mix of reaction is performed on a number of samples, plus additional sample. Prepare reaction mix work is done working inside the PCR, from Pre-PCR lab (clean room):
Kit "OneStep RT-PCR" RNasin and the primers reconstituted by hydration with ultra pure water and then aliquots are kept in a freezer at -20 °C.

To identify PRRS virus were used specific primers ORF7 area with the following sequence: PRRS-2 with 5′ - GCG GCG CAG CAC AAT ATG -3′ and PRRS WGT-4 with 5′ - GTA AAA AGA CAG CGA CTC TGG - 3′. (1)

Reagents before use they are defrost on ice. After defrost it homogenize less mix enzyme and RNasin then centrifuged few seconds (spin) at ≥ 10 000 rpm.

Master mix obtained is homogenize by pipetting. Pipette on ice in PCR tubes of 0.2 ml, 23μl Master Mix and 3μl ARN extracted.

**Amplification in thermocycler-Icycler**

Set the parameters of the following values:
- 48°C - 30’ 1x;
- 95°C - 10’, 1x;
- 95°C - 15’, 60 °C - 2’, 40x;
- 72°C - 7’ 1x;
- 4°C - ∞

Agarose gel electrophoresis is a common method of determining the size of PCR products.

On a sheet of Parafilm put 3-4 μl loading buffer (loading buffer) then add 10 μl of each sample.10 μ loading it from the mixture stirred in the wells of the agarose gel.

The electrophoresis tank add 12 μl, 10mg/ml EtBr in migration buffer in the anode (EtBr migrate from "+" to "+").

**Electrophoresis**
The amplified product is then detected by agarose gel electrophoresis

**Electrophoresis analysis**

Fragments of known size are placed into the first lane and a fraction of the PCR product is placed into consecutive lanes. Then, current is applied and the DNA is allowed to migrate.

The gel is stained with a fluorescent dye (ethidium bromide) which will only bind to the DNA present in the agarose gel.

The gel is visualized with an ultraviolet light and documented in a photograph (1).
This figure is an example of a PRRSV PCR electrophoresis gel. A single band at 256 base pairs indicates a positive test result. Lane 1 is a 100 bp ladder, used to estimate the size of the PCR products. Lanes 2–4 and 6 are positive field isolates. Lane 5 is a negative field sample. Lane 7 is the positive control and Lane 8 is the negative control sample.

**Results and discussions**

When an outbreak occurs in the farms studied were examined by PCR a total of 18 samples of organs for detecting PRRS virus infection. The investigations were conducted following results shown in Table 1

<table>
<thead>
<tr>
<th>Category</th>
<th>Sample no.</th>
<th>Sample no. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>piglets born dead</td>
<td>3</td>
<td>3 (100%)</td>
</tr>
<tr>
<td>piglets</td>
<td>9</td>
<td>6 (66.66%)</td>
</tr>
<tr>
<td>young pigs</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>fat pigs</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>18</td>
<td>9 (50%)</td>
</tr>
</tbody>
</table>
Of the 3 samples from piglets born dead and 6 samples from piglets were obtained specific lanes of PRRS virus. The obtained results are presented in Fig. 2.

Fig. 2. PRRSV detection by RT-PCR in agarose gel electrophoresis

At 2 months after the outbreak of PRRS were investigated 7 samples organs from dead body: 1 dead body piglet aged 25 days, 4 dead body youth aged between 42-100 days, and 2 bodies fat pigs aged between 125-130 days. The results obtained are presented in Table 2, respectively (Figure 3).

<table>
<thead>
<tr>
<th>Category</th>
<th>Sample no.</th>
<th>Sample no. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>positive</td>
<td>negative</td>
</tr>
<tr>
<td>piglets</td>
<td>1</td>
<td>1 (100%)</td>
</tr>
<tr>
<td>young pigs</td>
<td>4</td>
<td>2 (50%)</td>
</tr>
<tr>
<td>fat pigs</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>7</td>
<td>3 (45%)</td>
</tr>
</tbody>
</table>
From PCR image is observed that specific lanes PRRS genome are weakly positive in young pigs and intensely positive at organ sample taken from the piglet.

At 6 months after infection were investigated 16 samples collected as follows: 2 dead body piglets aged 25 days, 9 dead body young pig aged between 65-95 days and 5 dead body fat pigs aged 115 -125 days. The results obtained are shown in Table 3 and Figure 4.

<table>
<thead>
<tr>
<th>Category</th>
<th>Sample no.</th>
<th>Sample no. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>positive</td>
</tr>
<tr>
<td>piglets</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>young pigs</td>
<td>9</td>
<td>2 (22.2%)</td>
</tr>
<tr>
<td>fat pigs</td>
<td>5</td>
<td>1 (20%)</td>
</tr>
<tr>
<td>Total</td>
<td>16</td>
<td>3 (18.8%)</td>
</tr>
</tbody>
</table>

From next PCR picture observe that were obtained specific lanes of PRRS genome as follows: strongly positive at youth samples and weakly positive at samples from fat pig.
Fig. 4. PCR results (6 months of outbreak)

Conclusions

RT-PCR has been used extensively for viral detection and diagnosis because it demonstrates high sensitivity and specificity.

When the outbreak PRRS from the 18 organ samples, in 9 samples was present PRRS genome.

At an interval of 2 months from the first gather from the 7 samples were obtained specific lanes PRRS genome positive intensive pig at samples taken from piglet and weakly positive in samples from young pig.

After 6 months of infection from 16 samples collected were obtained specific lanes PRRS genome strong positive at 2 samples of youth and weakly positive at one samples from fat pig.

Riscul pentru o fermă de a se infecta este direct proporțional cu mărimea efectivului, nerespectarea carantinei și frecvența introducerilor de noi animale mai ales în sistemul intensiv de creștere.

The risk of becoming infected farm is directly proportional to the size of the herd, with the quarantine absence and frequency of introducing new animals in farm.

Acknowledgments

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References

**Summary**

This paper presents results obtained after three series of broilers growth to the ground in the same shelter were monitored. Infections with *E. coli* were diagnosis in studied broilers at first 10 days of age and over, at 11-20 days of age. Cumulative mortality increased form one series to another in the first 20 days due not respecting principle “all in – all out” and growing more consecutive series of broilers in the same shelter.

**Key words:** *E. coli*, broilers, epidemiology

Raising broilers is an activity that does not require a lot of investments. Therefore it is an activity that small breeders devote. Knowledge of bacterial infectious diseases that occur in the first days of life in farming facilities is needed to help both large and small-scale livestock farmers (2, 3).

*E. coli* septicaemia is spread across the globe. Disease produces economic losses through morbidity and mortality as well as the costs of treatment (1, 4, 6).

**Materials and methods**

In a poultry farm were followed three sets of broilers. The facility had one shelter with a capacity of 12,000 chicks per production cycle. Chicks were growth on the ground, from stocking time until delivery at 21 days of age.

The shelter was stocked with a number of 4,500 broilers Ross 308 hybrid per production cycle. Breeders were from Hungary and Italy.

When establishing epidemiological surveillance in the farm were broilers at different ages, as follows:
- 4,500 at 1 day old;
- 5,000 at 14 days old;
- 4,000 at 21 days old.

During the study were conducted clinical examinations, pathological and laboratory exams.
Broilers were daily monitored, following parameters: morbidity and mortality; co-infections; daily gain; and daily feed intake.

Chick’s delivery was made after each series has reached the age of 21 days.

**Epidemiological Surveys**

Epidemiological investigation aimed to highlight the aspects listed below:
- Broilers source;
- The principle “all-in all-out”;
- Providing feed stocks for the duration of chicks growth;
- Broilers health.

Broilers at different ages were growth in the same shelter, in pens equipped with heat lamps, as follows:
- In the first 10 days were accommodated 1,000 broilers/heat lamp/pen;
- In the next 10 days pens of two heat lamps were merged in order to increase chick’s space.

**Clinical and Pathological Examination**

Clinical examinations were performed daily. Were mainly followed signs that showed a change in the general condition, as well as the presence of respiratory, digestive or nervous symptoms.

Pathological examinations were performed at least once per day. Were examined at least 10 bodies for each series studied. On days when the mortality percentage increased, most of the bodies were examined. Gross lesions were observed for each body. According to their specificity, samples were taken for bacteriological examination.

**Bacteriological Examination**

Bacteriological examinations were performed in the Laboratory of Bacterial Diseases, belonging to the Infectious Diseases and Preventive Medicine Timisoara Department. For isolation of *E. coli* the bone narrow sampling were cultured on usual media 24 hours at 37°C. All samples were collected from chikens with specific lesions.

Bacterial identification was based by cultural and morphological aspects, tinctorial affinity and biochemical properties. For highlighting the morphological appearance and tinctorial affinity, Gram stained was made.

Biochemical properties of the strains were established by highlighting their behavior on selective and special media and with API 20 E test. Antimicrobials susceptibility by disc diffusion method was also tested.

As a result of bacteriological examinations performed, mortality causes were established, as well as the best therapeutic approach.
Results and discussions

Epidemiological Examination Results

Chicks were imported, and the transport was achieved in good conditions. The epidemiological examination pointed out that the principle “all-in all-out” was not applied in the shelter where chicks were growth, even if the shelter was clean, disinfected and microclimate was properly. Electrical installations were in working order.

In such a farm and stocking way the principle “all-in all-out” cannot be respected, so that there is continuous risk of the emergence of infectious diseases. 

Ventilation is achieved with speed controllable fans. Air inlet is through oblique-opened windows and through the door. This creates air currents that cause chicks crowding in warmer areas of the enclosure.

Mortality losses were recorded and analyzed. Their synthesis is shown in Table 1.

Table 1

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Series 1 (n=4500)</th>
<th>Series 2 (n=5000)</th>
<th>Series 3 (n=4000)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Deads</td>
<td>Deads</td>
<td>Deads</td>
</tr>
<tr>
<td>1</td>
<td>18</td>
<td>28</td>
<td>16</td>
</tr>
<tr>
<td>2</td>
<td>21</td>
<td>26</td>
<td>26</td>
</tr>
<tr>
<td>3</td>
<td>19</td>
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<td>21</td>
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<td>4</td>
<td>16</td>
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<tr>
<td>5</td>
<td>12</td>
<td>16</td>
<td>14</td>
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<tr>
<td>6</td>
<td>9</td>
<td>11</td>
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<td>7</td>
<td>11</td>
<td>15</td>
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<td>8</td>
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</tr>
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<td>9</td>
<td>5</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>10</td>
<td>7</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td>Total 1-10</td>
<td>126 (2.8%)</td>
<td>160 (3.2%)</td>
<td>132 (3.3%)</td>
</tr>
<tr>
<td>11</td>
<td>8</td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td>12</td>
<td>7</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>13</td>
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<td>10</td>
<td>11</td>
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<tr>
<td>14</td>
<td>3</td>
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<td>15</td>
<td>3</td>
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<td>16</td>
<td>4</td>
<td>7</td>
<td>5</td>
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<td>17</td>
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<td>18</td>
<td>2</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>19</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>20</td>
<td>2</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Total 11-20</td>
<td>41 (0.9%)</td>
<td>60 (1.2%)</td>
<td>64 (1.6%)</td>
</tr>
<tr>
<td>Total/series</td>
<td>167 (3.71%)</td>
<td>220 (4.4%)</td>
<td>195 (4.67%)</td>
</tr>
</tbody>
</table>
By analyzing data presented in Table 1, Figures 1 and 2 respectively, it is observed that in the three series of broilers were two peaks in the registered dead chicks number: in the first few days and in days 10-12.

At the age of 10 days the highest mortality rate was met in the third series, in which already 3.3% of chicks had died. The other two series had similar values: 3.2% in the second series and 2.8% in the first series.
In the next interval (10-20 days), the highest mortality rate was again recorded in the third series. Mortality value was 1.6%. Values in the other two series were lower: 1.2% in the second series and 0.9% in the first series.

For the entire holding period cumulative mortality of broilers is shown in Fig.2. Values were as follows: 4.87% for the third series, 4.40% for the second series and 3.71% for the first series.

By comparing those values it can be observed an increase from one series to another. This can be explained on the one hand by increased of microbial environment, and on the other hand by not respecting the “all-in all-out” principle.

Pathologic and Clinical Examination Results

In the first days of life (2-10 days), during clinical examinations have been noticed some symptoms such as: depression, horiplumation, dyspnea, diarrhea with white feces, etc. Symptoms reduced on the third day after administration of florphenicol, recommended by antibiogram.

Necropsy performed in the first days of life revealed mainly: unabsorbed vitellus, hepatic dystrophy, enlarged bladder (Fig. 3). In some chicks was also observed pulmonary congestion, uric acid and urate deposits in the ureters.

Necropsy later allowed highlighting the gross lesions of fibrinous polyserositis. Fibrinous polyserositis lesions (Fig. 4) were present in several examinations. This lesion allowed us to suspect the diagnosis of *E. coli* septicaemia. Diagnosis was confirmed by bacteriological examination.

Fig. 3. Dystrophic lesions in the liver
In the range 11-20 days during clinical examination were observed depressed chicks, with horiplumation and loss of appetite. At necropsy in this period the fibrinous polyserositis lesions and liver dystrophy were striking. After the treatment with florphenicol recommended by antibiogram, clinical signs disappeared. Subsequent liver lesions were not found, similar to those seen during this period.

All tests carried out revealed that these mortalities were due to E. coli septicaemia.

Between days 11-20 during necropsy the lesions that drew attention were those of fibrinous polyserositis and very rare cases with gout.

**Bacteriological Examination Results**

After bacteriological examination was performed, were isolated several strains of E. coli. Isolated strains were tested to determine antimicrobials susceptibility.

Highest sensitivity was observed to florfenicol (30 μg), gentamicin (50 μg), lincospectin (10 μg), enrofloxacim (5 μg) and ciprofloxacim (10 μg). Strains were moderately sensitive to amoxicillin-clavulanic acid, novobiocin and resistant to tetracycline, doxycycline, erythromycin and spectinomycin.

Analyzing the results obtained from the series of broilers growth in the same shelter, without respecting the “all-in all-out” principle, it is found that although seemingly mortality losses are not high, yet they are growing from one series to another.

This is due to shelter’s microbial environment growth, as well as growth in bacterial aggressiveness, mainly E. coli, revealed by multidrug-resistance of the isolated and tested strains.
It is known that the infection caused by *E. coli* in birds is a conditional disease (1, 5, 6). In our case it has been present in each set of broilers studied.

Conclusions

In the series of broilers studied (growth on the ground, in the same housing system, in order to be sold at the age of 21 days), were identified and were predominant *E. coli* infections, both in the first 10 days of life and in the range 11 to 20 days of life.

Cumulative mortality increased over time, from one series to another, due to not respecting the “all-in all-out” principle, and rearing in the same housing system of multiple consecutive series of broilers.

References

1. Decun, M. Infectiile colibaciliare la animale, Ed. Ceres, Bucureşti 1986
PREVALENCE OF *TOXOPLASMA GONDII* AND INTESTINAL PARASITES IN STRAY AND HOUSEHOLD CATS IN WESTERN ROMANIA

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Summary

To establish parasitic infestations in cats in Western Romania 605 samples were examined.

Seroprevalence of *Toxoplasma gondii* infection was determined using the ID-VET multispecies ELISA kit. Of all examined cats 361 (42.5%) had specific anti-*Toxoplasma* IgG antibodies, as follows: stray cats - 239 of 318 (75.15%), household cats - 122 of 287 (42.5%). The overall seroprevalence was higher in senior cats (> 7 years old, 84.29% of 126), than in adult cats (1.2 – 6.5 years old, 67.09% of 313) or in young group (< 1 year old, 26.5% of 166), and higher in male (67.2% of 311) than in female cats (51.7% 0f 294).

The prevalence of intestinal parasites was determined by the saturated NaCl solution coprological method. Of the 605 faecal samples in 191 (31.57%) were identified different species of parasites. In stray cats the prevalence was 33.01% (105 of 318) and in household cats the presence of intestinal parasites was identified in 29.96% (86 of 287). Parasitic species identified were: *Toxocara* spp. (11.4%), *Cystoisospora* spp. (6.61%), *Ancylostoma/Uncinaria* spp. (6.44%), *Taenia* spp. (5.62%), *Trichocephalus* spp. (1.32%) and *Toxoplasma-like* (0.16%).

Key words: *Toxoplasma gondii*, intestinal parasites, cats, Western Romania

Toxoplasmosis is a protozoosis caused by a coccidia belonging *Toxoplasmidae* family. Toxoplasmosis causes economic losses in the livestock sector through abortion, embryonic mortality or non-viable fetuses. Intermediate hosts are various species of birds and mammals, including humans and cats are the definitive hosts (11, 12). The serological surveillance indicated that *T. gondii* infection is widespread in farm animals. The most susceptible species are sheep, goats and pigs, and than leporidae, horses, cattle, and poultry (2, 3, 7). Among pets, cats. prevalence ranged from 2.1% in Guangdong, China, to 89.3% in Colombia and in dogs from 3.7% in the Czech Republic to 88.5% in Mato Grosso, Brazil (6).

The prevalence of toxoplasmosis vary depending on the living conditions of animals being raised in cats reared free who can hunt small mammals and birds and is much lower in animals raised only in the home, without access to the outside. In farm animals, the prevalence is higher where animals have direct
contact or indirectly, via animal feed, with cats (6). Seroprevalence of *T. gondii* infection varies from one country to another, but also within countries from one region to another, or even within the same city. These differences depend on the degree of civilization and culture of the human population. Feeding cats with controlled heat cooked food or commercial food can greatly reduce the incidence of this disease. Conversely, lack of food forces the predators to hunt to survive and thus continue the life cycle of the parasite.

**Materials and methods**

During 2007 - 2011, 605 biological samples were collected from cats from Arad, Timis and Caras-Severin counties from western Romania. Serological and fecal samples were from 318 stray cats and from 287 household cats, male (*n* = 311) and female (*n* = 294), aged between two months and 13 years, divided in juvenile (<1 year old, *n* = 166), adults (1.2 - 6.5 years old, *n* = 313) and senior (> 7 years old, *n* = 126) categories. From all these cats were collected individual fecal and blood samples and were processed in the laboratory of Parasitology and Parasitic Diseases of the Faculty of Veterinary Medicine Timisoara.

**ELISA**

For serological tests, blood was collected by cephalic venipuncture. Sampling was conducted in special tubes with clotting activator. The clot formed was removed from the tube, and the serum was kept in a freezer until assayed. After thawing of serum, a dilution of 1:10 was used. Serum samples were examined by indirect ELISA method using ID-VET kits Screen Multi-species (ID-VET Innovative Diagnostics, France) for specific IgG anti-Toxoplasma antibody. The 96-well plate is lined with *Toxoplasma gondii* antigen P30, and the antigen-antibody complex is formed with a conjugate of peroxidase added. The plate was analyzed in the microplate reader at 450 nm wavelength.

The optical densities obtained by reading the plate were interpreted according to the following formula: \( S / P = (\text{OD sample} / \text{OD MP}) \times 100 \). Values \( S / P \) of 200% was considered high positive, values between 50 and 200% were positive, between 40 to 50% were doubtful and values below 40% were considered negative.

**Coproscopical examination**

Faecal samples were collected in individualized special tubes from cats defecating places. In some cases it was necessary to isolate the animals and to use some laxative products.

Flotation method using sugar solution has the same technique as the Willis method, except that, in place of saturated sodium chloride solution, was used a mixture of 300 ml of water, 500 g sugar and 6.5 g of phenol crystals (1). The samples were microscopically (10x and 40x) examined.
Results and discussions

In studied cats, the overall seroprevalence was 59.67%. The highest seroprevalence was obtained in Arad County - 66.82% (139/208), followed then by the Caras-Severin County - 62.5% (75/120) and the lowest seroprevalence was in Timis County - 50.9% (141/277).

The results by age, sex and living conditions are shown in table 1 and fig. 1. The seroprevalence in senior cats was higher (84.92%) than in juvenile cats (26.5%). Also seroprevalence in males was higher (67.2%) than in females (51.7%). Greatest influence on the seroprevalence of *Toxoplasma gondii* infection had living conditions of cats. The stray cats seroprevalence was 75.15% (239/318) and in household cats was 42.5% (122/287).

<table>
<thead>
<tr>
<th>Cats population</th>
<th>Male</th>
<th>Female</th>
<th>Juvenile</th>
<th>Adult</th>
<th>Senior</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stray</td>
<td>137/173 (79.19)</td>
<td>102/145 (70.34)</td>
<td>33/87 (37.93)</td>
<td>142/165 (86.06)</td>
<td>64/66 (96.97)</td>
<td>239/318 (75.15)</td>
</tr>
<tr>
<td>Household</td>
<td>72/138 (52.17)</td>
<td>50/149 (33.55)</td>
<td>11/79 (13.92)</td>
<td>68/148 (45.94)</td>
<td>43/60 (71.66)</td>
<td>122/287 (42.5)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>209/311 (67.2)</td>
<td>152/294 (51.7)</td>
<td>44/166 (26.5)</td>
<td>210/313 (67.09)</td>
<td>107/126 (84.92)</td>
<td>361/605 (59.67)</td>
</tr>
</tbody>
</table>

Table 1

Seroprevalence of *Toxoplasma gondii* infection in cats related with sex, age and living conditions

Fig. 1. Graphical representation of *Toxoplasma* seroprevalence in cats
After examining feces in one of the samples were identified *Toxoplasma* like oocysts, but molecular tests were not performed to identify the species. Positive sample came from a four months stray cat, European breed and male sex. The cat was found on the street and brought to Faculty of Veterinary Medicine Timisoara Clinics for serological and coprological exams. By ELISA in this sample were identified also specific anti-*Toxoplasma* antibodies. In the other samples were not identified that type of oocysts.

Some faecal samples submitted other parasite species. Of the 605 samples examined were identified 191 (31.57%) positive fecal samples. The prevalence of intestinal parasites infestation was higher in stray cats (41.5%) than in household cats (20.55%). Coprological examination results are presented in Table 2.

**Table 2**

<table>
<thead>
<tr>
<th>Intestinal parasite species</th>
<th>Stray cats (n=318), n (%)</th>
<th>Household cats (n=287), n (%)</th>
<th>Total (n=605), n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Toxocara cati</em></td>
<td>47 (14.77)</td>
<td>22 (7.66)</td>
<td>69 (11.4)</td>
</tr>
<tr>
<td><em>Cystoisospora</em> spp.</td>
<td>19 (5.97)</td>
<td>21 (7.31)</td>
<td>40 (6.61)</td>
</tr>
<tr>
<td><em>Ancylostoma/uncinaria</em> spp.</td>
<td>31 (9.74)</td>
<td>8 (2.78)</td>
<td>39 (6.44)</td>
</tr>
<tr>
<td><em>Taenia</em> like</td>
<td>27 (8.49)</td>
<td>7 (2.43)</td>
<td>34 (5.62)</td>
</tr>
<tr>
<td><em>Trichocephalus</em> spp.</td>
<td>7 (2.2)</td>
<td>1 (0.34)</td>
<td>8 (1.32)</td>
</tr>
<tr>
<td><em>Toxoplasma</em> like</td>
<td>1 (0.3)</td>
<td>-</td>
<td>1 (0.16)</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>132 (41.5%)</strong></td>
<td><strong>59 (20.55)</strong></td>
<td><strong>191 (31.57%)</strong></td>
</tr>
</tbody>
</table>

From the percentages shown in Table 1, we see the influence of various factors on the prevalence of the disease. It appears that *Toxoplasma gondii* infection was more common in senior cats, and less common in juvenile who began to hunt.

The results of this study can be explained primarily by the life of animals, but also their diet and living conditions. Cats who came from rural areas and were maintained at homes or farms where they can hunt small animals have shown an increased prevalence of *Toxoplasma gondii* infection. Free access of cats from Romania and thus increase the risk of dispersion of eliminated oocysts makes that
Pastures, feed stores and animal shelters to be easily contaminated with *Toxoplasma gondii*.

Around the world, the results are the most diverse. The level of seropositivity (by ELISA) increases with age: 22% in cats under one year and 80% in those over 10 years. From 9 to 46% of pet cats from Europe and the United States, present at the serological test, a previous exposure to the parasite, while the toxoplasmosis seroprevalence in Asia has been estimated between 6 to 9% (5). In Belgium, 2% of cats aged under 12 months had anti-*Toxoplasma* antibodies, and 44% of cats about seven years were positive (4). Miro et al. (2004) identified, in Spain, a seroprevalence of 36.9% in stray cats, 33.3% in farm cats and 25.5% in household cats (10).

Iovu et al., in 2009, conducted a retrospective study on the prevalence of *T. gondii* infection in animals in Romania (8). Regarding the prevalence of toxoplasmosis in cats data were varied. Serological, Mircean et al., 2008 identifies a prevalence of 57.7% by ELISA ImmunoComb, in cats in central and north-western Romania. The authors obtained a prevalence increasing with cats age so: in kittens under six months age - 29.4%, of cats aged between six months and two years - 50%, of cats aged 2:10 years - 69.1% and in cats over 10 years - 66.6% (9). In an attempt to identify *T. gondii* oocysts in the feces of cats, Titilincu et al., 2008, did not get any positive sample and Mircean et al., 2008 identifies a single positive sample of 253 tested (0.33%) (9, 13).

*Toxoplasma* infection of cats in the three studied Counties is important because of the possibilities of disease transmission in livestock animals and humans by oocysts spread in the environment.

**Conclusions**

The overall seroprevalence of *Toxoplasma gondii* infection in cats, in western Romania, was 59.67%.

In Timis County, the prevalence of toxoplasmosis in cats was 50.9%, in Arad was 66.82% and in Caras-Severin was 62.5%.

Seroprevalence of *Toxoplasma gondii* infection in cats was influenced by age, sex, and area of origin of the cat.

At one cat were identified *Toxoplasma*like oocysts and IgG specific anti-*Toxoplasma* antibodies which points us toward the diagnosis of toxoplasmosis.

In the fecal samples were identified associated infections with: *Trichocephalus* spp., *Toxocara cati*, *Cystoisospora* spp., *Ancylostoma/Uncinaria* spp. and *Taenia* spp.
References


VARIATION IN CRUDE PROTEIN CONTENT OF SOME FEED MATERIALS

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Summary

To ensure protein proper needs, animal body must be provided with a balanced diet in crude protein. Quality of feed materials used in feeder industry may vary, depending on various factors.

In this study we evaluated variation in crude protein content of some feed materials, during 2003-2007. Of the 125 samples of soybean meal crude protein content ranged between 33.13% to 48.98%, with an average of 42.2393%. In samples of sunflower meal (made from partially shelled seeds) (n = 100), crude protein showed fluctuations between 28.26% and 37.7%, with an average of 34.3882%. Fish meal samples (n = 100) had a crude protein content between 52.95% and 74.09%, the average being 63.3338%.

Compared to reference values cited in the literature (Reference tables ©INRA-AFZ) studied samples showed higher mean values in sunflower meal (34.3882% vs. 33.4%) and lower averages in soybean meal (42.2393% vs. 45.32%) and in fish meal (63.3336% vs. 65.36%).

These fluctuations may influence the relationship between efficiency of feed rations and animal productions performance.

Key words: crude protein, soybean, sunflower, fish meal

Protein sources most commonly used in animal feed are soybean meal, sunflower meal and fish meal. These components often come in the structure of compound feeds for different animal species. The quality of materials is very important for quality control of the finished product. Crude chemical composition of these materials must be constantly controlled to highlight possible variations that can influence the quality of compound feed recipes. Any variation in feed chemical content can cause nutritional imbalances in animals and thus, decrease of animal production.

Establish of variation in crude protein content for the main feed sources used in animal nutrition may be an aid to feed manufacturers or farmers in the composition of a balanced recipes that meet the nutritional requirements of animals. An adequate fed animal is a healthy animal that ensure increased production for the entire economic life.
Materials and methods

For this study 325 feed samples collected in 2003-2007 were assayed. Were collected 125 samples of soybean meal, 100 samples of sunflower meal, partially dehulled and 100 samples of fish meal. Feed samples came from different farmers and compound feed manufacturers from Arad and Timis counties. Samples were assayed in the Laboratory of feed chemical analysis from Nutrition, Feed and Agronomy Department, Faculty of Veterinary Medicine Timisoara. For the determination of crude protein was used Kjeldahl method and apparatus used were InKjel - D - 40599 and Behrotest - WD - 40. Feed samples were dried to constant weight, ground and then were weighed, using 1 g of each sample.

Results and discussions

The results are presented in Table 1 and Figure 1.

<table>
<thead>
<tr>
<th>Feed /year</th>
<th>Crude protein content (mean, %)</th>
<th>Sunflower meal</th>
<th>Soybean meal</th>
<th>Fish meal</th>
</tr>
</thead>
<tbody>
<tr>
<td>2003</td>
<td>34.39</td>
<td>42.23</td>
<td>63.33</td>
<td></td>
</tr>
<tr>
<td>2004</td>
<td>34.39</td>
<td>42.23</td>
<td>63.33</td>
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<tr>
<td>2005</td>
<td>34.39</td>
<td>42.23</td>
<td>63.33</td>
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<tr>
<td>2006</td>
<td>34.39</td>
<td>42.23</td>
<td>63.33</td>
<td></td>
</tr>
<tr>
<td>2007</td>
<td>34.39</td>
<td>42.23</td>
<td>63.33</td>
<td></td>
</tr>
<tr>
<td>Crude protein mean</td>
<td>34.39</td>
<td>42.23</td>
<td>63.33</td>
<td></td>
</tr>
</tbody>
</table>

During those five years the content of crude protein showed variations in all three types of feed materials. Sunflower meal showed the lowest values of crude protein (33.72%) in 2003. Values were growing up in 2006 (table 1), following that in 2007 will fall again (33.96%). Mean crude protein content during the 5 years was 34.39%, representing a value of nearly 1% higher than that cited in the literature (33.4%) (Reference tables © INRA-AFZ) (5).

Smaller values (40.8%) of crude protein content in the soybean meal were recorded in 2005. From 2003 to 2005 values have been declining (table 1), in 2006 crude protein content increased to 43.53% and in 2007 dropped again to 42.95%. Mean crude protein (2003-2007) was 42.23% representing a value with 3.09%
lower than the average value cited in the literature (45.32%) (Reference tables © INRA-AFZ) (5).

Crude protein content in fishmeal submitted close relative values in the first three years of the study (table 1). In 2006, the value increases sharply to 65.04% and will then drop to 63.38%, in 2007. The average percentage of crude protein content in fish meal (63.33%) was with 2.03% lower than indicated in the INRA tables (65.36%) (Reference tables © INRA-AFZ) (5).

Fig. 1. Variation of crude protein content in comparison with to mean values from INRA tables

From the results presented it can be observed an increase in crude protein content than the mean cited in the literature only in sunflower meal; in soybean meal and fishmeal values being lower. Also, there is a higher amount of crude protein in all three types of feed raw materials in 2006 than all other studied years. It is known that environmental factors, temperature, rainfall regime, soil quality influence the chemical composition of feed plants. It may be that this year environmental factors influenced favorable the crude protein content of feed materials studied. For fish meal, crude protein content can vary depending on the type of raw material used.
These results highlight the importance and necessity of testing the chemical composition of feed before being administered to animals or before feed recipes or compound feeds being made for different categories of animals. Real content of crude protein in feed may differ from the values cited in the literature, this thing representing a starting point of various feed deficiency or excess that can disrupt health and animal productions. Quality diet is maintained through quality feed materials, emphasizing the absolutely necessary chemical analysis of the raw ingredients.

Similar studies have been achieving in other countries. Bista and Shrestha (2000) evaluated the crude protein content of soybean meal from different regions of Nepal and found significant differences between the values obtained in the East (40.3%) than those from the central area (39%) of Nepal (3). Also in Nepal, Tiwari et al. (2006) showed variation in crude chemical composition of different feed materials and the importance of these variations on diets of livestock animals (6). Relandreau (2013) identified a mean crude protein content of 48% in soybean meal, with variations between 44 and 51% (4). Azar (2012) found a crude protein content in sunflower meal of 30% and Ariyawansa (2000) identify a variation of crude protein of fish meal between 68.0% and 73.1%, depending on the type of raw material used (1, 2).

On livestock animals production and productivity are extremely important in justifying economic life, and these factors are very easily influenced by the quality of animal feed. Therefore, by maintaining a high standard of quality feed is maintained high level of animal productivity.

Conclusions

In all three types of feed materials were recorded variations in crude protein content.

Content of crude protein showed higher mean values in sunflower meal and lower content in soybean meal and fishmeal than reference values cited in the literature (Reference tables © INRA-AFZ).

Knowing the precise crude chemical composition of the feed is very important for the preparation of balanced feed recipes.

Feed quality can influence productivity of livestock animals.

References


OBSERVATIONS ON THE EVOLUTION OF AN OUTBREAK OF AVIAN INFECTIOUS BURSAL DISEASE

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Summary

Avian infectious bursitis (BIA), also known as Gumboro disease, is an infectious disease, common in chickens and characterized by inflammation of the bursa of Fabricius. Specific prophylaxis provides active immunization of livestock for this purpose are used inactivated vaccines in breeding hens and live vaccines in broilers administered in several ways, including "in ovo".

Research aspect were performed in a broiler farm in southwest of Romania, on a flock of 10,500 chickens of Cobb hybrid, raised from the ground, vaccinated with a vaccine containing strain 20512 Winterfield administered "in ovo" in incubation, to embryos of 18 days at a dose of 0.05 ml / embryo.

The disease was diagnosed by the anatomopathologic examination performed twice weekly and confirmed by RT-PCR reaction and by ELISA. Anatomopathological examination provided data on the presence of certain specific lesions BIA and cumulative mortality ranged between 0.34% and 1.46%.

RT-PCR was detected very virulent virus BIA prototype, but has not been detected vaccine strain. By ELISA was detected antibody BIA with minimum titers to 19 days and significant titers at 33 days of age.

The results obtained show that the BIA has evolved broiler flock monitored because the vaccine used administered "in ovo" provided a partial immunity.

Key words: Avian infectious bursitis, chickens, vaccine.

Avian infectious bursitis (BIA), also known as Gumboro disease, is an infectious disease, common in chickens and characterized by inflammation of the bursa of Fabricius. The disease is known across the globe, but in 1986, after the appearance of strains "very virulent" European importance of the disease has increased due to significant economic losses include: morbidity, mortality, reduced feed conversion rate, reduced weight growth associated with state immunosuppression which favor the occurrence of secondary bacterial infections.

The BIA prophylaxis measures based on general and specific measures these being applied in breeding farms and broiler farms.
Specific prophylaxis of provides active immunization of livestock for this purpose are used inactivated vaccines in breeding hens and live vaccines in broilers. Live vaccines are biological products prepared from live strains with different degrees of attenuation: attenuated, attenuated intermediate, intermediate, intermediate plus and hot, which may be administered in several ways.

**Materials and methods**

The researches were performed in a broiler farm in southwest, on a herd of 10,500 chickens of Cobb hybrid, raised from the ground. The evolution of the outbreak was monitored using the following exams: epidemiological, clinical, pathological exam and laboratory. In epidemiological surveys have been investigated sources of infection and cumulative mortality was monitored developments and clinical examination was followed by the presence of symptoms, and other symptoms.

Pathological examinations were performed twice weekly to identify specific lesions BIA and other infectious diseases. BIA virus was identified using the method of real-time amplification SybrGreen for virus detection and discrimination BIA. Viral RNA was extracted with QIA amp Viral RNA Mini Kit (Qiogen 52,906), and discrimination against virulent virus, the vaccine strain was done with primers Thiveyrtt (ELIN). Final identification was performed spectrophotometrically amplifier by gel electrophoresis Mx 300Sp with MxPROVO1/2007 program.

BIA specific antibodies were detected by immunoassay test, indirect variant with Flock Chek kit Antibody Elisa IBO-XR product IDEXX Laboratories. For this purpose blood samples were collected from chickens at 19 days (11 samples) and 33 days (18 samples).

**Results and discussions**

BIA was diagnosed initially on specific lesions detected by pathological examinations performed twice weekly. Epidemiological surveys conducted as a longitudinal survey type, identified secondary sources of infection that can maintain the farm virulent virus, which after populating infects chickens. Cumulative mortality calculated weekly had variable limits, ranging between 0.34% and 1.46%, increasing progressively from the third week after populating. Clinical examination revealed the chicken's ill symptoms of BIA, but the large number of chickens daily examination did not allow the entire flock. Pathological examination provided data on the presence of certain BIA. Thus, in week III and IV lesions were found specific to this disease: dehydrated cadavers, feathers cretaceous agglutinated with feces diarrhea, petechiae and
ecchymoses the pectoral muscles and legs, the bursa of Fabricius increased in volume with lesions catarrhal or catarrhal hemorrhagic and yellowish containing fibrin, nephrosis with urate in the ureters and catarrhal enteritis. The presence of these lesions corresponds to cumulative mortality increased from week III.

Serological examination performed by ELISA, indirect variant provided data on the evolution of the immune response against BIA. Thus, at 19 days, chicks, 7 samples did not detect antibodies to the virus BIA, samples were negative, and 4 samples were detected low titers of antibodies. Average antibody titer value of 453 was not protected adequately the flock of chickens.

At the age of 33 days, the chicks, all serum samples were detected with antibody titers BIA significant titer is the 9519 average.

The method of amplification in real time RT-PCR, the burses taken from cadavers 21 to 28 days, virus was detected BIA, classified as very virulent pathotype, but was not detected attenuated vaccine strain existing vaccine composition.

Results from the monitoring show that broiler flock BIA vaccine administered “in ovo” provided a partial immune protection. Very virulent pathotype virus BIA kept in the farm chickens infected by secondary sources or because low titers. Very virulent pathotype virus farm BIA kept chickens infected by secondary sources or because low titers.

Clinical and pathological exams reported the presence of BIA, in the herd, but could not be established disease morbidity. Also, the frequency of the specific lesions of disease was reduced which argues for a relatively small number of chickens unprotected by the vaccine administered.

The presence of very virulent pathotype BIA virus was demonstrated by real-time PCR bursa of Fabricius lesions in confirming specific infection. Absence of attenuated strains in vaccine is harder to explain, although vaccination was made “in ovo”.

Serological examination results show a reduced immune response, at the age of 19 days, confirmed by BIA appearance at the age of 21 days. Significant titers, the antibodies BIA, at the age of 33 days post infection suggests an immune response, also confirmed the presence of disease.

Necropsy revealed and secondary bacterial infections (colisepticemia, mycoplasmosis) consecutive postinfectious immunosuppression. The results show that vaccination "in ovo" does not provide good protection of chickens against very virulent pathotype infection with the virus BIA and research in this area should be expanded.

Conclusions

BIA has evolved from broiler after the ages of 3 weeks since vaccination "in ovo" induce an immune response, which did not protect chickens against very virulent pathotype.
Cumulative mortality was variable values, and the lesions were present in
the bursa Fabricius and kidney, the latter being very virulent pathotype specific.

Very virulent pathotype was detected by RT-PCR in real time version, but
not the vaccine strain was detected.

At the age of 19 days average antibody titer was 453, not providing
adequate protection against virulent pathotype.
At the age of 33 days average antibody titer was 9519, which shows a post
infectious immune response.

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RESEARCH ON THE COLICINS PRESENCE IN THE APEC STRAINS

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Summary

E. coli strains classified in APEC pathotype cause extraintestinal infections in poultry which have the situs of penetration in the organism, respiratory mucosa.
This includes pathotype strains most commonly employed in serogroups O1, O2 and O78, which have many virulence factors: fimbriae, siderophores, outer membrane proteins, complement resistance, endotoxins, exotoxins, and colicins.
Plasmids encoding synthesis colicins are Col IV, V and Col BM so far been identified about 20 colicins.
Were studied 146 strains based on phenotypic and genotypic characteristics, were within the APEC pathotype.
Colics synthesis was studied by the standard method using ROW strain (strain-sensitive colicin).
At APEC strains that produced colicins around colonies appeared crisp inhibition zones due to diffusion in agar, which inhibits the growth ROW strain.
Research has shown that synthesis colicins is common in APEC strains.
Thus, a total of 111 strains (76.02%) produced colicins and 35 APEC strains did not produce colicins.

E. coli strains classified in APEC pathotype cause extraintestinal infections in poultry with the situs of penetration into the body, respiratory mucosa (1).
This includes pathotype strains most commonly employed in serogroups O1, O2 and O78, which have many virulence factors: fimbriae, siderophores, and outer membrane proteins, complement resistance, endotoxins, exotoxins, and colicins (1, 5).
APEC strains containing and conjugates plasmids responsible for multiple antibiotic resistances (R plasmid) and plasmids governing synthesis colicins respectively plasmids Col IV, V and Col BM (2, 3).
E. coli produces 20 colicins employed recently in exotoxins group that if APEC strains are considered virulence factors (1, 3).
Research has followed colicins presence at APEC strains isolated from outbreaks of colibacillosis in broilers.
Materials and methods

Were studied 146 strains based on phenotypic and genotypic characteristics, were within the APEC pathotype.  
Strains were isolated from broiler cadavers from many farms with colisepticemy injuries.  
Based on biochemical properties (lactose fermentation, behavior and environments TSI and MIU API20E system), isolates were classified into species E. coli and fixation on Congo red on the iss gene and identification, fim H and omp PCR were included in APEC pathotype (1, 3).  
Subsequently, strains were tested for antibiotic behavior towards being thus established and main rezistotypes.  
Colicins synthesis was studied by the standard method using ROW strain, which is a strain of E. coli, strain-sensitive to colicin (4).  
Strains were grown in broth and nutrient agar seeded drop in Petri plates, each plate 5 strains. After 24 hours, cultures obtained (macrocolonii) were killed with ultraviolet (UV) exposure for one hour, the quartz lamp.  
Next, 0.1 ml of culture broth, strain ROW was built in 10 ml soft agar existing in a test tube at a temperature of 45 °C. After mixing, the tube contents were poured onto Petri dishes macrocoloniile killed and after 24 hour incubation at 37 °C was interpreted result.

Results and discussions

At APEC strains that produced colicins around colonies appeared crisp inhibition zones due to diffusion in agar, which inhibits the growth ROW strain.  
At APEC strains that produced colicins (colicinogene strains) around macrocoloniilor appeared crisp inhibition zones due to diffusion in agar, which prevented the development of stem colicins ROW. Zones of inhibition vary in size, being dependent diffusion colicin power products and the type of colicin developed.  
Colicin production research has shown that this property is common in APEC strains. Thus, a total of 111 strains (76.02%) produced colicin and 35 APEC strains did not produce colicin.  
Synthesis of colicins (bacteriocins) is governed by Col plasmids, plasmids conjugate widespread in strains of E. coli. May be able nonconjugativ, but with less frequency (1, 4).  
Recent research, molecular biology has shown that there are several types of Col plasmids such as Col IV, Col V, Col BM responsible for the synthesis of a number of 20 colicins, recently assigned group of bacterial toxins and, therefore, colicinele to serve proinfectios APEC strains (2, 3).  
The results obtained show a high frequency phenomenon strains colicinogemie at APEC, which can help increase the pathogenicity of these strains.
Standardized method for phenotypic method based on sensitivity to strain ROW colicine allowed identification APEC strains producing colicine, whose frequency is increasing, this phenomenon has been reported by other authors (2, 3, 5).

Recent research based on molecular biology tests have shown that there are several types of Col plasmids and thus several types of colicins the most active and best studied being colicin V, whose synthesis is iron-dependent (2, 3).

Studies on plasmid profile at APEC strains have shown that plasmids Col, conjugates were 4 types of genes: genes coding for self-replication, gene encoding colicin the gene responsible for immunity protein synthesis and plasmid transfer genes responsible Col. (1,2,3)

In a comprehensive study on colicins synthesis at APEC strains, MELHA MELATA et al. (2010) found that these strains frequently produce colicin V and contain conjugative plasmids Col IV and Col BM. They also demonstrated that EIT ABCD genes present in these plasmids were reported to serogrup strains belonging to O1 and O2, the genes encoding factors associated with the acquisition of iron.

Colicin synthesis, phenotypic highlighted at APEC strains tested, proved that a large percentage (76.02%) of strains producing these substances, suggesting the presence of conjugative plasmids Col type associated with other virulence factors.

Col plasmids are transferred by conjugation between strains of E. coli, this phenomenon contributes to the increasing number of pathogenic strains of bird populations. (1.3)

Conclusions

Colicins synthesis at APEC strains can be evidenced by phenotypic method using strain ROW.

Colicins synthesis was present in a large number of APEC strains studied, proving such high frequency of strains carrying colicinogenic (or Col) factors.

Colicins have pro infective effect, contributing to increased pathogenicity of APEC strains.

References


SEROSURVEY OF BABESIA INFECTION IN STRAY DOGS FROM TIMIS COUNTY

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Summary

Stray dogs are considered sentinels in the epidemiology of many infections including babesiosis as their lifestyle involves direct contact with ticks. Sampling was carried out between January 2011 and January 2013 in dog shelters from Timiș County. Serum samples from 213 dogs were assayed by indirect fluorescent antibody test Megascreen FluoBabesia canis and Megascreen FluoBabesia gibsoni, a commercially available antigen kit (MegaCor Diagnostic GmbH, Hörbranz, Austria) for the presence of anti-Babesia canis and anti-Babesia gibsoni antibodies. Overall the prevalence was 20.6 % (44/213) for Babesia canis and 2.3% (5/213) for Babesia gibsoni, respectively. No statistically significant association were found between the seropositivity of the infection and the age and gender of the dogs. The results of this study highlighted that the dogs without owner from Timiș County can be considered an important reservoirs in the epidemiology of Babesia infection.

Key words: Stray dogs, Babesia, IFAT

Canine babesiosis is a tick –borne disease produced by an intracellular protozoa manifested with hyperthermia, hemoglobinuria, various degrees of anemia and sometimes icterus. On the basis of their morphological appearance in the erythrocyte, the parasite is divided in two species: large specie - Babesia canis and small specie - Babesia gibsoni. Clinical signs are different from severe to mild symptoms depending on specie and immunological status of the dog (4, 12).

Seroepidemiological investigations can determine the current status of the disease and the risk factors for animal population. Stray dogs are considered sentinels in the epidemiology of many infections including babesiosis as their lifestyle involves direct contact with ticks. Serological tests may detect asymptomatic carrier dogs and also chronic infections, in which the level of parasithaemia may be low and not detectable in stained blood smear or antibodies level in a certain moment.

The IFAT and ELISA techniques are considered highly sensitive and moderately specific for detection of antibodies to Babesia parasites in dogs (5, 15).

The aim of this study was to determine the seroprevalence of B. canis and B. gibsoni infection in stray dogs from several shelters of Timiș County.
Sampling was carried out between January 2011 and January 2013 in dog shelters from Timiș County. Samples consisting of peripheral blood were collected from 213 stray dogs in EDTA sterile vacutainers. Plasma/serum was obtained after sedimentation and stored at -20°C until further processed. Samples were then assayed by indirect fluorescent antibody test Megascreen FluoBabesia canis and Megascreen FluoBabesia gibsoni, a commercially available antigen kit (MegaCor Diagnostic GmbH, Hörbranz, Austria) for the presence of anti-*Babesia canis* and anti-*Babesia gibsoni* IgG antibodies. The slides containing fixed blood infected with *B. canis* (Megacor, Germany) and *B. gibsoni* were stabilized at room temperature 1 hour before use. Serum samples were diluted 1:32 in PBS and 20 µl were pipetted onto the circumscribed areas. The slides were incubated in the humid chamber at 37°C for 30 minutes. After washing, 1 drop of Dog-IgG-FITC Conjugat was added to each delimited area and then incubated for another 30 minutes. The slides were then washed and 2 drops of mounting fluid was added between the slide and the coverslip.

The slides were examined under a fluorescence microscope using 40x objective.

The samples that showed clearly fluorescence in the field were considered positive after comparison with positive and negative controls (Fig 1). The negative reactions didn’t present any florescence.

Data were analyzed using Minitab 16 Statistical Software (Minitab, Pennsylvania) and differences were considered statistically significant when \( P \) values were less than 0.05.
Results and discussions

Based on serological examination of 213 samples, 44 (20.6%) were positive for *B. canis* and 5 (2.3%) for *B. gibsoni* antibodies, respectively. Among *B. canis* positive stray dogs, 18 (40.9%) were females and 26 (59.1%) were males. Out of 5 *B. gibsoni* positive samples 3 (60%) were females and 2 (40%) were males. No statistically significant association were found between the seropositivity of the infection and gender of the dogs.

In a previous study, in a larger area (three counties) the prevalence of antibodies for *B. canis* in clinically healthy dogs, with owner, was lower (19.8%) than in the current survey, but the difference was not significant (p>0.05) (8). The higher prevalence (20.6 % vs. 19.8 %) may be due to the lack of acaricid treatments in stray dogs. Consequently, the clinically manifested *Babesia* infection rate may be higher in stray dogs, but in the absence of treatment can lead to death of ill dogs. Also, the number of survival dogs achieving proper antibody titer can be low.

In the same study hunting lifestyle and living in the environment of rural areas are considered favoring factors. The number of seropositive dogs with *Babesia canis* was higher (44.1%) in hunting dogs compared with companion and kennel dogs and in those living in rural areas (28.4%) compared with the ones from urban areas (15.4%) (8).

All over the world the serological surveys by IFAT for the prevalence of *B. canis* revealed prevalence from 35.7% to 66.9% in Brazil (11, 13), 14.1% in France (3), between 17% (14) and 34% (4) in Italy, between 9.9% (10) and 13% (6) in Albania and 5.7% in Hungary (7).

Seroprevalence of *B. gibsoni* in dogs was investigated in a small number of studies, especially in the United States and Japan. In a study from North Carolina stray and kennel American Pitt Bull Terrier and American Staffordshire Terrier dogs were investigated for the presence of antibodies revealing a prevalence of 5.84% (21/359) in stray dogs and 14.47% (22/149) in kennel dogs (1). Also, in Australia, Jefferies et al. reported the presence of *B. gibsoni* in 14 (9.27%) out of 151 dogs American Staffordshire Terriers, American Pit Bull Terriers and Japanese Tosa tested in IFAT and PCR (2, 9).

The considerable seroprevalence values for *B. canis* and *B. gibsoni* infection found in this study, demonstrated the relatively high exposure rate of stray dogs to *Babesia* infection. Therefore, these dogs can be considered as important reservoirs for other animals and tick vectors.

Conclusions

In serological examination of 213 blood samples from stray dogs 44 were positive for *B. canis* (20.6%) antibodies. *B. gibsoni* antibodies were found in 5 (2.3%) dogs sampled.
No statistically significant association was found between the seropositivity of the infection and gender of the dogs.

Dogs without owner from Timiș County can be considered important reservoirs in the epidemiology of *Babesia* infection.

**Acknowledgements**

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PREDVALENCE OF RESPIRATORY VIRUSES AND BACTERIA IN
ROUTINELY MEDICATED, NON-VACCINATED FATTENING
STEERS

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Summary

Viruses and bacteria causing respiratory disorders observed in two beef cattle farms in Central Serbia were investigated. Samples of discharge from the nasal mucosa were taken for the isolation of the cause of viral and bacterial origin, from clinically diseased fattening steers, Simmental race, six months old. Using the method of Real Time PCR (RT-PCR), we try to detect genome of Bovine Herpes Virus type-1 and Bovine Viral Diarrhea Virus, which predisposing bacterial infection. Isolation of bacterial organisms from nasal discharge on artificial culture media was determined by aerobic cultivation. In our examination, we don't determine the genome sequences of BHV-1 and BVD virus. The most common bacterial findings were Pasteurella multocida, Staphylococcus albus, Streptococcus viridans, Str. pneumoniae, Bacillus sp., E. coli, Naisseria cattarhalis, Klebsiella pneumoniae and Micrococcus sp. Susceptibility was investigated by the disk-diffusion method and revealed that the most efficient antibiotics against bacteria were Florfenicol (Floron), Ampicillin, Amoxicillin and Amoksiklav.

Key words: bronchopneumonia, viruses, bacteria, aerobic cultivation, RT-PCR, susceptibility

The complex etiology of respiratory diseases of cattle (Bovine Respiratory Disease Complex - BRDC) viruses causes primary lesions, primarily in the respiratory tract [bovine herpesvirus type-1 (BHV-1), parainfluenza-3 virus (PI-3), bovine respiratory syncytial virus (BRSV), and bovine viral diarrhea virus (BVDV)]. Lesions caused by direct destruction of the epithelial cells, where it creates an inflammatory exudate, which is an ideal growing medium for bacteria. The aforementioned viruses lead to the dysfunction of neutrophils, lymphocytes, and most important of alveolar macrophages (1). The most common bacterial pathogens were isolated Mannheimia (Pasteurella) haemolytica, Pasteurella multocida, Histophilus Somni (Haemophilus Somnus) and Mycoplasma spp. (5). Mannheimia haemolytica, Pasteurella multocida and Histophilus Somni are common inhabitants of naso-pharyngeal mucosa, but not lung. Mannheimia haemolytica serotype 1 is the main cause of BRD's, because it is the most common and virulent serotype, but the cause may be some other serotypes determined.
from 16 serotypes of the same bacteria. Clinical signs are monitored to identify potentially infected cattle from the BRCD: level (number) respiration, character expiratory/inspiratory; fulfillment of the rumen, anorexia, nasal and ocular discharge and depression (2). Cattle in which observe these symptoms are separated into classes for therapy, for further examination (follow-up of clinical signs and rectal measurement of body temperature). A decision on the mode of therapy and is recorded in the outpatient protocol, and relies mainly on an elevated rectal temperature (above 39.7 °C). The data show that only 75 % of vaccinated animals a protective immunity, even when they are immunized with vaccines finest. For these reasons, most of the research efforts are directed towards the study of causes, their genotype and phenotype modifications, pathogenesis and immunological processes that develop during the BRDC (6). Woolums et al. (9) found that the BRDC was number one cause of morbidity and mortality in fattening calves (561 facilities in 21 United States of America countries - 12.8 % of a cattle herd). About 12.57 % of cows affected with BRDC were treated with medication, but 0.75% died. Economic losses of the livestock industry, as a consequence of the appearance of BRDC, expressed through the rate of morbidity and mortality, the cost of prevention and treatment with drugs, reduced productivity and value of cattle carcasses (7). Production losses (costs not included death of cattle) in the EU at a level of approximately € 576 million per year (3).

**Materials and methods**

Experimental animals were beef cattle, 5 months old, both sexes, Simmental race. Steers are the property of "Kotlenik-promet" Ltd., Lađevci, from two farms, located in Central Serbia, near Čačak. From anamnesis and clinical examination on the day of sampling, diagnosed the severe symptoms which justify suspicion of BRDC (lose weight following a loss of appetite, visibly rapid breathing, lung auscultation tightened breathing, cough progresses to sound relatively dry, ocular and nasal discharge visible as either serous or yellow and viscous, depression and a progressive fever - rectal temperature above 40.1 °C).

Samples of discharge from the nasal mucosa were taken using sterile swabs and test tubes for the isolation of the cause of viral and bacterial origin, from clinically diseased and suspected animals breed in same facilities. All samples were shipped in a hand in the refrigerator within one hour after sampling in the accredited microbiological laboratory of Veterinary Specialist Institute "Kraljevo".

Isolation of bacterial organisms on artificial culture media was determined by aerobic cultivation, with subsequent biochemical identification and determination of the isolated strains (Methods for isolation and identification. Guide for the laboratory diagnosis of bacterial, viral and parasitic diseases, 1984).

Susceptibility testing of isolated bacterial strains to antibiotics and sulfonamides (antibiogram) was performed using the disk diffusion method, according to Kirby-Bauer procedure.
Real Time PCR (RT-PCR) method
a) Determining the genome of IBR/IPV - methods number M235.

The genome of the virus IBR/IPV was extracted using the QIAamp DNA Mini Kit (Qiagen, Germany). PCR reaction was performed using the Maxima™ Probe qPCR Master Mix (Fermentas, Lithuania), the Real Time PCR machine MX3000P Stratagene, according to the protocol described in the OIE Manual, Chapter 2.4.13. b) Determination of the genome BVDV, the number of method M236. BVDV genome was extracted using the QIAamp Viral RNA Mini Kit (Qiagen, Germany). RT-PCR reaction was performed using the Superscript III Platinum® One-Step Quantitative RT-PCR System (Invitrogen, USA), the Real Time PCR machine MX3000P Stratagene, according to the protocol described by Baxi et al. (2006).

Results and discussions

Results of the microbiological tests and susceptibility testing presented in tables below (1-2).

Our test results were very similar to those of other authors (5), and are important for the diagnosis and determination of therapy for experimental and other cattle herds tested. Predominant isolated bacteria were Pasteurella multocida, and we tested her susceptibility to antibiotics and sulfonamides, showed in table 2. There were no genome sequences BHV-1 and BVD virus in any of the examined nasal swabs of beef cattle, by RT-PCR. From data presented in table 2, we can conclude that the most efficient antibiotics against Pasteurella multocida isolates were Florfenicol, Amoxicillin and Amoksiklav (100 % isolates sensitive).

<table>
<thead>
<tr>
<th>BACTERIAL AND VIRAL AGENTS</th>
<th>Farm 1</th>
<th>Farm 2</th>
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<td>IS 1b</td>
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<tr>
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<td>+</td>
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<td>Pasteurella multocida</td>
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<td>Streptococcus pneumoniae</td>
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<tr>
<td>Klebsiella pneumoniae</td>
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<td>Micrococcus sp.</td>
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<tr>
<td>IBR/IPV</td>
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<td>BVDV</td>
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Table 1

Microbiological tests of samples of nasal swab from beef
Table 2

Susceptibility testing of *Pasteurella multocida* isolates to antibiotics and sulfonamides (antibiogram)

<table>
<thead>
<tr>
<th>ANTIBIOTICS</th>
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<td>R</td>
<td>R</td>
<td></td>
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</tr>
<tr>
<td>Gentamycin</td>
<td>I</td>
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<td>I</td>
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<td>I</td>
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<tr>
<td>Penicillin</td>
<td>R</td>
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<td>R</td>
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<tr>
<td>Oxytetracycline</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Florfenicol (Floron)</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td></td>
<td>S</td>
<td>S</td>
</tr>
</tbody>
</table>

IS 1* - no. of isolates; S - Sensitive; I - Intermediate sensitivity; R - Resistant.

Conclusions

Powerful instrument of modern epidemiology was genotyping and phylogenetic analysis of antigenic types and subtypes of viral and bacterial pathogens, based on the comparison of the nucleotide/amino acid sequences of certain fragments of the genome in order to determine similarities/differences and their classification. The most common bacterial findings were *Pasteurella multocida*, despite the facts that the *Mannheimia haemolytica* predominantly determined worldwide. Interactions with other bacteria and viruses are unclear yet. Most efficient antibiotics against *P. multocida* isolates were Florfenicol, Amoxicillin and Amoksiklav. Conduct more extensive tests presented to enable more effective prevention of BRDC, while reducing the cost of medical treatment and economic losses, more effective and more efficient vaccination of cattle and the rational and responsible use of synthetic antibiotics.

Acknowledgements

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References


THE DYNAMICS OF BLOWFLIES OF CALLIPHORA, LUCILIA AND PROTOPHORMIA GENERA DURING 2005 IN NORTHERN TIMISOARA

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Summary

Blowflies are commonly shiny flies with metallic colouring, often with blue, green, or black thoraces and abdomens, and which laid their eggs on fresh meat or wounds, causing myiasis in livestock. During 2005 the blowflies of Lucilia, Calliphora and Protophormia genera were monitored between April 20 and October 30. For Calliphora genus 6 population peaks were recorded. First capture was achieved on April 27th, at a 13.1°C mean air temperature, 13.8°C mean soil temperature and 73% relative humidity, and the last one on October 25th, at a 11.0°C mean air temperature, 11.4°C man soil temperature and 78% relative humidity, respectively. For Lucilia genus 6 population peaks were recorded, with May 03rd the first capture date, and October 11 the last capture date. For Protophormia genus were only 4 population peaks recorded. First capture was performed on May 18th at a mean air temperature of 21.0°C, a mean soil temperature of 20.3°C and 64% relative humidity, and the last one on September 11, at a mean air temperature of 21.1°C, a mean soil temperature of 27.0°C and 75% relative humidity.

Key words: dynamics, blowflies, 2005, northern Timisoara.

Blowfly strikes represent an important challenge in many countries in which sheep breeding is well developed. In example, during `90s, in New Zealand, the annual losses totaled almost 70 million dollars (5, 7, 10).

It was observed that all over the world the fly-strike prevalence and duration of the season in which blowfly strikes appear increased significantly. If these cases occur in the southern hemisphere in a wave form from November till February (6), in countries with temperate climate from boreal hemisphere these attacks are more frequently in August and September and rarely in October (1, 2, 3), but they can spread throughout the hot season, starting with June (4).

Data from autochthonous literature concerning the population dynamics of blowflies from Calliphoridae family are scarce. That’s why this study aimed to establish this dynamics during the year of 2005 in northern Timisoara.
Materials and methods

The study has been carried out in the park of Parasitic Diseases discipline of the Faculty of Veterinary Medicine Timişoara.

Blowflies were captured by the mean of a parallelepiped-like trap as was described by Morariu and Morariu (9) in a previous work. Two traps were placed and maintained two consecutive days in the park, between 10 AM and 06 PM, starting with April 20 and finishing with October 31st. The month of August was not monitored because of objective reasons.

Relative humidity and minimal and maximal values of temperature were measured in the sampling day. For the other days the meteorological data were collected from INMH, Timişoara branch.

At the end of each day of study the traps were covered with nylon bags, and then, in the Parasitic Diseases’ Laboratory, the blowflies were killed by the mean of cotton swabs soaked with chloroform introduced in bags.

The examination was performed under a stereomicroscope, and several of morphological characteristics were taken into account to genera identification: eyes position, antennae shape and color, thoracic macrochaetae, color of basicosta, wings nervatures, and color and hairiness of thoracic stigmas (anterior and posterior).

Results and discussions

Figures 1-3 show the evolution of monitored blowfly populations during 2005. For Calliphora genus the first catch was done on April 27th, at an air average temperature of 13.1°C, a soil average temperature of 13.8°C, 73% relative humidity and 11.4 hours of sunshine, respectively (Figure 1), but temperatures constantly exceeded 15°C before of this trapping.

The first population peak was recorded on May 03rd having only 18 flies trapped (Figure 1). As temperature increase and the relative humidity decrease a second peak with 108 individuals collected was noticed, 31 days apart from the first one, at an air average temperature of 17.5°C, a soil average temperature of 27.3°C, 48% relative humidity and 13.2 hours of sunshine.

During the month of July, two population peaks were observed recorded: first one on July 01st, with 276 trapped blowflies, and a second one 28 days apart (on July 29th), with the maximal load of caught blowflies (482 individuals).

Two more population peaks were observed when monitoring was resumed after the break from August. On September 06th 341 blowflies were captured, and on October 04th (28 days apart) only 113 individuals were trapped. After that, a severe decline in number of caught blowflies was noticed. At the last population peak the following parameters were recorded: 17.2°C air average temperature, 17.8°C soil average temperature, 78% relative humidity and 9.1 hours of sunshine, respectively.
Nevertheless, the last capture was done on October 25th, after a long cool and wet period.

Therefore, for blowflies of *Calliphora* genus six population peaks were recorded, but the constancy between peaks is very surprisingly. Thus, in 2005, the length of life cycle for individuals of this genus was of 28-31 days.

For *Lucilia* genus, the 2005 year began with a first catch on May 03rd (2 blowflies), at an air average temperature of 19.2°C, a soil average temperature of 20.8°C, 64% relative humidity and 11.2 hours of sunshine (Figure 2). But, after this date, temperatures decreased very much and the relative humidity increased, which drastically reduced the number of captured blowflies. Therefore, the first population peak (36 individuals) was noticed late, only on July 01st, when the air average temperature was of 17.5°C, the soil average temperature was of 24.3°C, the relative humidity was of 50% and 8.6 hours of sunshine.

The second population peak was registered also late, 28 days apart, period in which there were days without trapped *Lucilia* blowflies.

The third population peak coincide with the maximal peaks of 2005 (242 blowflies), and was achieved on July 20th, at an air average temperature of 22.3°C, a soil average temperature of 28.1°C, 59% relative humidity and 13.3 hours of sunshine, except the lack of information about the month of August.
On September 06th the fifth population peak (101 trapped blowflies) was observed, and the last one was recorded on October 04th, with only 7 blowflies captured, at an air average temperature of 17.2ºC, a soil average temperature of 17.8ºC, 78% relative humidity and 9.1 hours of sunshine. The last trapped individual was noticed on October 11, at an air average temperature of 12.7ºC, a soil average temperature of 16.6ºC, 73% relative humidity and 7.3 hours of sunshine.

Thus, six population peaks were recorded for Lucilia genus, and the life cycle had 21-28 days length.

For Protophormia genus, 2005 starts inauspiciously (Figure 3). First capture was registered only on May 18th, at an air average temperature of 21ºC, a soil average temperature of 20.3ºC, 64% relative humidity and 4.8 hours of sunshine, respectively.
The first population peak was noticed late, on June 01st, with only 21 blowflies trapped. However, in the same month, on June 24th, the second peak (92 blowflies collected) was recorded.

The maximal peak was observed on July 15th, with 149 collected individuals, at an air average temperature of 20.8°C, a soil average temperature of 24.5°C, 74% relative humidity and 8.5 hours of sunshine. As the weather went warmer, the number of collected blowflies increased, but the real maximal population peak couldn’t be assessed during the month of August because no monitoring activities were performed in this month.

A new peak (102 blowflies) was registered on September 11, which was also the last one for this genus. Due to the significant cooling of the weather the number of collected Protophormia blowflies sharply decreased and the last capture (only 2 blowflies) was noticed on October 02nd.

Thus, for Protophormia genus only four population peaks were recorded during 2005. The decreasing trend was evident in this genus, including also the number of individuals collected at the highest peak. The length of the life cycle was difficult to establish. The lowest range was 21 days, during the summer season, but the highest one had have not enough landmarks to be recorded. However, it could be presumed that exceeded 30 days.

When compared with the year of 2004, the results were different: for Calliphora genus six peaks were recorded, while for Lucilia and Protophormia genera five peaks were noticed (8).
Conclusions

For *Calliphora* and *Lucilia* genera six population peaks were registered, while *Protophormia* one recorded only four peaks.

The highest population peak was seen on July for all monitored genera.

The shortest life cycle interval was noticed for *Lucilia* blowflies (21 to 28 days).

References

EPIDEMIOLOGICAL AND SEROLOGICAL RESEARCHES IN AN AVIAN INFECTIOUS BRONCHITIS OUTBREAK

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Summary

Our researches were conducted in a poultry farm, from Timis County, specialized in consumption eggs production, in which BIA (avian infectious bronchitis) has evolved after approximately two months after laying onset. During the researches, the epidemiological, clinical, necropsy and laboratory exams were conducted, in order to elucidate the disease developing mode and to establish an appropriately diagnosis. The epidemiological survey, carried out on the farm, confirmed that the BIA outbreak occurred as a result BIA virus penetration into the farm through secondary sources. The qualitative eggs changes were: eggs with soft shell, deformed eggs, depigmented eggs, small eggs, sometimes the egg yolk were missing and other times was mixed with the egg whites. Egg production quantitative changes were represented by a decreased egg production, averaging 32% in each hall. The serological exam has confirmed the BIA’s presence in the farm; the geometric means and titer groups, highlighting through post-infectious immune response, in dynamic, respectively the seroconversion phenomenon. The serological exam results have proved a correlation between the antibody titers, expressed in D.O. and the laying curve evolution.

Key words: avian infectious bronchitis, epidemiologic exam, serological exam

Avian infectious bronchitis is a coronavirosis with high infectivity, which affects chickens of all ages, with a higher mortality in young, being in the recent years, widespread in poultry farms, representing a constant threat to intensive poultry (1, 6, 7). Morphoclinical manifested by respiratory, renal, genital and enteric disorders (4, 5, 6, 7). The trade with poultry has contributed to the worldwide spread of several variant strains of the BIA virus. These strains are actually new genotypes with increased virulence (velogene) capable of causing disease in chickens, youth and adult, due to their tropism for the respiratory tract, kidney, oviduct and intestine. Due to the antigenic differences between the vaccine strains and variant strains, vaccinations, in the hens flocks youth and adult, does not immunological cover the birds vaccinated against the circulating strains in the chicken farms.

Most of the times, the vaccine strains belonging to serotype does not protect the birds from infection with other serotypes of BIA. Thus, this situation occurred frequently in poultry farms, especially in adult hens raised for...
consumption eggs or for breeding (2, 3, 6). The economic losses produced by the BIA, are large and, in some cases, cannot be quantified precisely thanks to the intervention of some factors represented by: breeding technology, hygiene, feed, and the presence of parasitic and infectious diseases associated to BIA. Such a situation has evolved into a poultry farm in Timis County, specializing in the production of consumption eggs in which BIA has evolved around two months after the laying onset.

Materials and methods

In one poultry farm, from Timis County, specialized in consumption eggs production, at the age of 35 weeks of the laying hens, quantitative and qualitative changes occurred in the production of eggs. To establish the disease diagnosis, the following tests were performed: epidemiological, clinical, pathological, bacteriological and serological.

The epidemiological exam. This test was carried out as epidemiological investigation, pursuing the following objectives: identifying the infection source, the percentage of laying dynamic, detection of predisposing factors and mortality. Also was examined the immunological situation of the hens flock, the preventive treatments developed and other diseases evolution.

The serological exam. This exam was conducted in order to confirm BIA suspicions. In order to perform this test, blood samples were taken from chickens, in a randomized order, as follows:
- R1 - at the age of 37 weeks (10 samples of H13, 10 samples of H14, and 10 samples of H15)
- R2 - at the age of 39 weeks (10 samples of H13, 10 samples of H14 and 10 samples H15)

After the expression, the sera, it was decanted, in Ependorf tubes, numbered and stored in a freezer until the time of serological examination. The specific antibodies were detected by ELISA (Enzyme Linked Immunosorbent Assay) kit using FlockChek® Infectious bronchitis Antibody Test Kit, provided by IDEXX Laboratories provided, Inc. (46). The serological tests were performed at S.N. Pasteur Institute S.A. Bucharest.

Results and discussions

The examinations performed in the farm in which BIA have appeared and evolved, has provided important results with practical utility regarding the evolution of this disease in laying hens farms. The BIA outbreak, which occurred in this farm, has evolved into a time when there was a resurgence of the disease in the country.

The epidemiological exam: This exam was carried out under a epidemiological investigations whose objectives were outlined above. The first changes in egg production occurred starting with 25.12.2008, initially in a hennery and after that, it appeared also in the other two, at the chicken age of 35 weeks.
The pullets have been raised on the ground until the age of transfer, after which they were moved into three halls with pyramid batteries 8400 chickens in each hall.

The pullets were imported from Slovakia at the age of one day being Issa-Brown hybrids. They have received growing technology, animal health and feeding according to the hybrid requirement. Until the transfer age, the pullets were vaccinated against Newcastle disease and infectious bursitis and received preventive treatment against coccidiosis at the age of 19-21 days. Also, after each vaccination, was performed an antistress treatment with an antibiotic (Enrofloxacin) and multi-vitamins in the drinking water.

In the ground growing, the hygiene and feeding conditions were appropriate and infectious diseases were not registered.

At 16 weeks of age, the pullets were transferred into three halls, as follows: hall 13 (8640 heads), hall 14 (8640 heads), hall 15 (4540 heads). The pullets were housed in 3 levels pyramid cages, 3 in each box. After the transfer, they were fed and subjected to a light program correlated with their age, according to Issa-Brown hybrid performance.

At the time of transfer, or at 16 weeks of age, the pullets were vaccinated against avian smallpox, infectious bronchitis, laying drop syndrome and NewCasstle disease.

For avian pox prophylaxis, the Nobilis ® Ovo-Diphtherin vaccine was used, containing WP strain of avian pox virus, which was administered by stick method (intradermal). The Nobilis ® IB + ND + EDS vaccine is use for immunoprophylaxis in avian infectious bronchitis, Newcastle disease and the egg drop syndrome. Is an inactivated vaccine with formalin and emulsified (oil adjuvant). In the vaccine composition comprises: Massachusetts serotype, lentogene strain 30 Clone and a stain of Egg drop syndrome. The vaccine is administered to chickens between the ages of 16 and 20 weeks by intramuscular or subcutaneous way, in dose of 0.5 ml / bird.

From the analysis of the immunological situation result that for BIA, there was only a single vaccination with the recalled vaccine. Manufacturer's recommendations and hybrid Issa-Brown growing technology, recommends a prior vaccination with a live vaccine respectively Nobilis ® MA5, which contain a cloned strain of the Massachusetts serotype, at the age of 6 weeks, either in drinking water or by aerosols. This vaccination with a live vaccine produces an immune response that is amplified by the second vaccination (booster) made with the inactivated vaccine.

In the mentioned farm, the biosecurity and general prevention measures have been partially applied, existing in the farm, a frequent movement of people and vehicles. Also, in the farm, chickens are raised for meat, which are sold live at different ages, and some of them are slaughtered.

Another important observation is that the car that transport the eggs to stores enters in the farm till the birds halls, and the eggs boxes are reused returning from the shop back to the farm.
In the period in which BIA have evolved, no transmitted or non-transmitted diseases were reported and the mortality was within the permissible limits of technology.

After the disease manifestation, on every hall was given an antibiotic (Enrofloxacine) and a multi-vitamins complex in the drinking water for 7 days to prevent secondary bacterial infection and for stimulating the hens organism.

The epidemiological investigation has confirmed that the source of BIA virus infection was represented by several secondary sources, such as vehicles, egg boxes and people. The disease progressed in this farm because the hen’s immunological status was not represented by protective titers of serum antibodies because there was a single hens vaccination. Also during this period, in the country was isolated the serotype variant 4/91, considered to be the serotype with the greatest virulence and his antigenic structure is significantly different from vaccine strains.

The qualitative eggs changes: At the eggs exam was found discoloration, shape, size and weight changes. The eggs with common sizes were very common but they had soft shell, or the eggs were normal but distorted (asymmetric twist), followed in frequency the depigmented eggs with normal or small dimensions. The small eggs remained as frequency on a long period of time (fig. 1, 2 and 3).

At the eggs with these changes the yolk was missing or the egg content was not delimited. Changes in content were present also on normal sized eggs.

The quantitative eggs changes: the quantitative changes represented by lower egg production had a different evolution in the hens flocks of the three halls. The first quantitative changes occurred at the age of 35 weeks, after 25.12.2008.

Fig.1. Eggs with modifications

Fig. 2. Small and depigmented eggs
The egg production was daily recorded and the laying percentage was daily calculated for each hall in order to track the laying curve progress. These results were interpreted and presented in tables 1 and 2 and in figure 4.

Table 1

<table>
<thead>
<tr>
<th>Hall</th>
<th>Week</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>VI</th>
<th>VII</th>
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<td>93-95</td>
<td>95-92</td>
<td>91-85</td>
<td>85-84</td>
<td>84-85</td>
</tr>
<tr>
<td>14</td>
<td></td>
<td>95-94</td>
<td>94-67</td>
<td>66-92</td>
<td>93-94</td>
<td>94-87</td>
<td>87-87</td>
<td>87-88</td>
</tr>
<tr>
<td>15</td>
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<td>95-89</td>
<td>87-69</td>
<td>64-78</td>
<td>78-87</td>
<td>88-89</td>
<td>89-90</td>
<td>90-91</td>
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</tbody>
</table>

Table 2

<table>
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<th>Week</th>
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<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>VI</th>
<th>VII</th>
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<tbody>
<tr>
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<td>96-70</td>
<td>70-93</td>
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<td>95-92</td>
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<td>78-87</td>
<td>88-89</td>
<td>89-90</td>
<td>90-91</td>
</tr>
</tbody>
</table>

The period in which the laying curve was tracked ranged between 25.12.2008 and 10.02.2009 and is divided in 7 weeks and the results are weekly presented weekly. After the onset of laying, the laying curve have progressively increased being similar
with the potential laying curve of the hybrid Issa-Brown, until the outbreak of the disease.

In the 13 hall before the disease outbreak occurred the egg production, was 96%, after which the egg production have daily decreased, reaching 70% in the sixth and seventh day after the disease onset. In the following days, the egg production began to increase, reaching after nine days 95%. In the fourth week of the period, the egg production decreased again, being 84% and 85% in the last two weeks.

In hall 14, before the disease outbreak occurred, the egg production was 95%, and since 29.12.2008 has declined progressively, reaching ten days after the onset, 67%. In the coming days have rise again, all gradually being averaged 93.64% in the fourth week. Since the fifth week of the period, the egg production decreased again being averaged 87.25% in the sixth week and 88% in the seventh week.

In hall 15, the egg production began to decline starting with 26.12.2008, reaching the lowest percentage, 64% in III week, respectively at 16 days after the disease onset. Subsequently, the laying percentage grew more slowly than in the other two halls, but has not suffered a second fall, so that in the last two weeks the laying percentage was 90%. Analyzing these results, we observe that the egg production reduction was quite severe, the laying percentage dropping with a rate from 96% to 64%. This decrease associated with the qualitative changes is similar to the information existing in literature, including the one in our country.

![Fig 4. The laying curve evolution](image)

The significant egg production dropping was the consequence of the intervention of variant serotype 4/91, of the virus BIA identified in several farms from our country. This serotype identification was made after investigations in
several farms by IDSA Bucharest. Throughout this period of 7 weeks, respectively, from the age of 35 weeks until the age of 42 weeks, on the farm chickens number, no changes were observed and no appearance of “old actually” considered to be characteristic by some authors (7, 34, 37). To reduce the effect of BIA, on birds, a treatment was established, referred to above, which prevented the occurrence of secondary bacterial infections and which clinically recovered the chickens number.

The serological exam: The serological exam was conducted in order to confirm BIA in that specific farm, this disease being epidemiologically and clinically suspected.

This examination results were performed through ELISA are presented in the tables and the geometric average of specific antibody titers, expressed in OD are shown in figure 5. The obtained results were processed using a soft interpretation of that kit being played in each sample and actual groups. Subsequently, all through this soft, the data obtained were processed statistically being shown the arithmetic and geometric mean of the specific antibodies titers.

In 13 hall, on R1, the minimum titer was 2736 OD and the maximum titer was 41116 OD. The samples were divided into 8 titer groups (3-14), all samples were considered positive.

At R2, performed after 14 days, the minimum titer was 5497 DO and the maximum titer was 29303 OD, the samples were grouped into 9 titer groups (5-13), all samples were considered positive.

The geometric mean between the two harvests was approximately equal, as well as other results of this examination, which shows an old infection. On this hall, on which BIA first debuted, the disease is correlated with the dynamics of the immune response after vaccination.

In the 14 hall, at R1, the minimum titer was 11209 OD and the maximum titer was 22696 OD being identified 7 titers groups (8-16), all samples were positive. At R2, performed after 14 days, the minimum titer was 25995 OD and the maximum titer was 49472 OD, the samples were grouped only into two titers groups (15-16) which exemplifies the phenomenon of seroconversion, and all samples were positive. On this hall, the geometric mean R2 was 1.46 times higher than the geometric mean on R1, confirming also seroconversion.

In the 15 hall, at R1, the minimum titer was 8414 OD and the maximum titre was 30914 OD eight titer groups were identifies (6-16), all samples were positive.

At R2, performed after 14 days, the minimum titer was 8319 DO and the maximum titer was 27204 OD, the samples were grouped into 7 groups (6-14), all samples were positive. Also in this hall, the two geometric means between harvestings were approximately equal, as well as other data of this test, which indicates an older infection.

The results of the serological exam, besides that have confirmed the BIA diagnosis; they also revealed the presence of a post-infectious immune response. At all three halls, the minimum titers recorded at R1 were considered positive.
These high values were a consequence of infection rather than an immune response after vaccination (table 3, 4, 5, 6, 7, 8 and 9).

**Table 3**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Do</th>
<th>S/P</th>
<th>Titer</th>
<th>Titer group</th>
<th>Result</th>
</tr>
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<tr>
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<td>1.326</td>
<td>24878</td>
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<tr>
<td>2</td>
<td>0.993</td>
<td>1.038</td>
<td>16810</td>
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<tr>
<td>3</td>
<td>0.767</td>
<td>0.768</td>
<td>10398</td>
<td>7</td>
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</tr>
<tr>
<td>4</td>
<td>1.646</td>
<td>1.816</td>
<td>41116</td>
<td>16</td>
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<tr>
<td>5</td>
<td>1.015</td>
<td>1.064</td>
<td>17494</td>
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<tr>
<td>6</td>
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<td>0.991</td>
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<tr>
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<td>0.61</td>
<td>7187</td>
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<tr>
<td>8</td>
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<td>0.333</td>
<td>2736</td>
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<tr>
<td>9</td>
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<td>0.958</td>
<td>14791</td>
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<tr>
<td>10</td>
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Statistical interpretation:

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<tr>
<td>Max</td>
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**Table 4**

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<tr>
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<th>Titer group</th>
<th>Result</th>
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**The serological exam results Hall 14 - R1**

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### Table 6

**The serological exam results in H14 - R2**

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### Table 8

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In the recent years, in most European countries with intensive poultry, have emerged and rapidly disseminated variant serotype 4/91, which has a very high pathogenicity for both baby and for laying hens. This serotype variant has significant antigenic differences from the known serotypes used to produce vaccines. The presence of serotype 4/91, was confirmed in Romania, in several poultry farms. Infections with this serotype are followed by high intensity immune responses of antibody titers.

Due to these epidemiological and immunological situations, it requires the use of bivalent vaccines that contain the Massachusetts serotype and serotype 4/91, to provide a post-vaccination protection much wider and more efficient. These types of vaccines are corresponding to the protect type notion, which is different from the serotype notion, in the sense that immune protection is provided against several circulating serotypes.

Conclusions

The epidemiological survey carried out in the farm confirmed that the BIA outbreak occurred as a result of virus penetration in the farm, through secondary sources.

The hens have not received an immune response, protective after vaccination, because they were vaccinated against BIA, only once at the age of transfer.

The eggs quality changes were: the shell softening, deformation, depigmentation and small dimensions. Also the yolk was missing or the egg content was depigmented.
The quantitative changes in egg production were represented by lower egg production with an average of 32% in each hall.

Decreased egg production, appeared at the age of 35 weeks, due to hens, unprotected immunological, probably with variant serotype 4/91 BIA virus that entered into the farm through secondary sources.

The egg production has recovered, but never reached the level before the BIA emergence.

Throughout the evolution of BIA outbreak were reported other viral or bacterial diseases.

The established treatment have prevented secondary infection and clinically recovered the hens number.

The serological examination have confirmed BIA presence in that farm, the geometric mean and the titer groups are underlining a post-infectious immune response, in dynamic, respectively a seroconversion phenomenon.

Serological examination results showed a correlation between antibody titers expressed in R.O. and the laying curve evolution.

References

8. ***Poultry & Livestock Catalog and ELISA Technical Guide, IDEXX Laboratories, Inc
SEROPREVALENCE OF TOXOPLASMA GONDII INFECTION IN PIGS FROM ROMANIA EVALUATED BY IFAT, MAT AND ELISA

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2Université Paris-Est, École Nationale Vétérinaire d’Alfort, UMR BIPAR ANSES ENVA UPEC USC INRA, Maisons-Alfort, F-94704, France
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Summary

The aims of this study were: (1) to evaluate serological tests used for T. gondii antibodies detection in pigs and (2) to determine the seroprevalence of T. gondii antibodies in domestic pigs, reared in intensive system from Romania. Two “in house” methods, indirect immunofluorescent antibody test (IFAT), the modified agglutination test (MAT) and two commercially available ELISA kits (ID Screen Toxoplasmosis Indirect Multi-Species, ID.vet, France and Toxoplasma Microwell Immunoassay Kit, Safe-Path Laboratories, Carlsbad, CA) were compared for their sensitivity and specificity in detection of anti-T. gondii antibodies in naturally infected pigs using as standard cumulative seropositivity. Also, their agreement was evaluated by kappa statistic. A good correspondence of test results was observed. The best agreement was seen between IFAT and ELISA ID.vet ($k=0.89$). The IFAT has the higher sensitivity (97.4%) and ELISA ID.vet has the higher specificity (100%) and Jouden index ($J = 0.95$). The highest seroprevalence was obtained by ELISA ID.vet (43.6%), followed by IFAT (40.4%), ELISA Safe-Path (33%) and MAT (27.7%).

Key words: Toxoplasma gondii, IFAT, MAT, ELISA, domestic pigs.

Domestic pigs (Sus scrofa) are considered as an important source of T. gondii infection in humans (3) and acute toxoplasmosis has been reported in humans that have consumed uncooked infected meat from pigs (1). Most pigs acquire T. gondii infection postnatally by ingestion of oocysts from contaminated environment or ingestion of infected tissues of animals. Few pigs become infected prenatally by transplacental transmission of the parasite (3, 7, 15).

Many serological tests have been used to detect anti-T. gondii antibodies in pigs. The most commonly tests used were the modified agglutination test (MAT) (6), indirect fluorescent antibody test (IFAT) and commercial ELISA tests using whole-tachyzoite antigen (8, 11). The importance of knowing the epidemiological situation of T. gondii infection in pigs lies in the role that pork meat products play in public health. The purpose of this study was to evaluate four serological methods (two “in house” tests...
MAT and IFAT and two commercial ELISA’s) for detecting anti-\textit{T. gondii} infection and determine \textit{T. gondii} seroprevalence in pigs raised in intensive system.

**Materials and methods**

**Animals and serum.** In 2008, serum samples from 94 domestic pigs reared in intensive system from Romania were collected. All pigs were originated from Cluj county, north-west of Romania.

**Sero logical assays**

**Immunofluorescence antibody test (IFAT)**

The test used whole tachyzoites of \textit{T. gondii} from peritoneal exudates of mice infected intraperitoneally with the \textit{T. gondii} RH strain. The initial peritoneal exudate obtained from the mouse must be passaged every third or fourth day into fresh mice. The exudate for passage should be acellular as possible and must be free of bacterial contamination. It is diluted in antibiotic saline (to 500ml of sterile saline add 2ml of Heparin 5000units/ml and 5ml of penicillin/streptomycin solution) until the diluted exudate contains approximately $10^6$ organisms/ml. 0.5ml of this diluted exudate is inoculated intraperitoneally into fresh mice.

An anti-swine IgG fluorescein conjugated goat IgG fraction (Jackson Immuno research Laboratories Inc., catalog no. 114-095-003, lot 77747) served as conjugate. The cut-off of the immunofluorescence test was established at a dilution of 1:32. The test was carried out as described by Győrke et al. (2011).

**Modified agglutination test (MAT)**

The modified agglutination test (MAT) for the detection of \textit{T. gondii}-specific IgG antibodies was performed as previously described (4), using an antigen prepared from formalin- fixed whole RH strain tachyzoites. Each serum samples was serially twofold diluted. The threshold dilution was 1:6.

**Enzyme-linked immunosorbent assay (ELISA)**

Two different commercially indirect ELISA techniques for IgG detection were used: ID Screen \textit{Toxoplasmosis} Indirect Multi-species (ID.vet, France) and \textit{Toxoplasma} Microwell Immunoassay Kit (Safe-Path Laboratories, Carlsbad, CA).

The tests were performed according to the manufacturer’s instructions.

**Statistical analyses**

**Evaluation of test characteristics and level of agreement**

This study determined sensitivity (Se), specificity (Sp), Jouden index (J), positive and negative predictive values (PPV and NPV) of the IFAT, MAT, ID.vet and SafePath ELISA’s, for anti-\textit{T. gondii} antibody detection, by analyzing serum samples from 94 naturally infected pigs came from intensive system, by WinEpiscope software. As golden standard (comparative test) we used a cumulated seropositivity (CP), being considered positive those samples that were positive at least two of the four applied methods.

Also, agreement between the all four serological tests and CP was assessed by the calculation of kappa statistic value.
Kappa has values from -0.25 to 1, and interpreted as follows (17): $k \leq 0.20$ without consistency, $0.21 \leq k \leq 0.40$ poor agreement, $0.41 \leq k \leq 0.60$ moderate agreement, $0.61 \leq k \leq 0.80$ good agreement, $k > 0.80$ very good agreement.

Tests characteristics and level of agreement were performed by Win Episcope 2.0 program.

**Descriptive epidemiology**

Frequency, prevalence and its 95% confidence interval of anti-*T. gondii* antibodies, processed by IFAT, MAT, ID.vet and SafePath ELISA’s, were established. All statistics were performed using the EpilInfo 2000 software.

**Results and discussions**

Serum samples from 94 naturally infected pigs from intensive system, were tested by all four serological methods, for evaluation of test characteristics and level of agreement.

A good correlation was observed, 18 positive serum samples and 49 negative serum samples were obtained by all four methods. ELISA ID.vet (43.6%; 41/94; CI 95% 33.4-54.2) and IFAT (40.4%; 38/94; CI 95% 30.4-51.0) had the most positive results, while MAT had the fewest (27.7%; 26/94; CI 95% 18.9-37.8%) (Table 1). IFAT obtained the best Se (97.4) and NPV (98.2) and ELISA ID.vet obtained the highest Sp (100), PPV (100) and J (0.95) (Table 1).

Table 1

<table>
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<th>95% CI</th>
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<td>MAT</td>
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<td>27.7</td>
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<td>43.6</td>
<td>33.4-54.2</td>
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<td>Safe-Path ELISA</td>
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<td>33</td>
<td>23.6-43.4</td>
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<td>CP</td>
<td>39</td>
<td>41.5</td>
<td>31.4-52.1</td>
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</table>

Legend: CP - cumulated seropositivity (were considered positive those samples that were positive at least two of the four applied methods).

An optimum agreement, according with Petrie and Watson (1999), was obtained between IFAT and ID.vet ELISA ($k = 0.89$) and a moderate agreement was obtained between Safe-Path ELISA and MAT ($k = 0.47$). Excellent agreement was obtained between cumulated seropositivity (CP) and ID.vet ($k = 0.96$), IFAT ($k = 0.93$) tests and a good correlation was observed between CP and SafePath ELISA ($k = 0.73$), MAT ($k = 0.66$) (Table 2).
Table 2

<table>
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<td>Positive predictive value</td>
<td>94.9</td>
<td>64.1</td>
<td>100</td>
<td>74.4</td>
</tr>
<tr>
<td>Youden index</td>
<td>0.94</td>
<td>0.76</td>
<td>0.95</td>
<td>0.78</td>
</tr>
<tr>
<td>Kappa agreement</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CP</td>
<td>0.93</td>
<td>0.66</td>
<td>0.96</td>
<td>0.73</td>
</tr>
<tr>
<td>IFAT</td>
<td>0.63</td>
<td>0.89</td>
<td>0.7</td>
<td></td>
</tr>
<tr>
<td>MAT</td>
<td></td>
<td></td>
<td>0.62</td>
<td>0.47</td>
</tr>
<tr>
<td>ID.VET ELISA</td>
<td></td>
<td></td>
<td></td>
<td>0.69</td>
</tr>
</tbody>
</table>

Legend: CP - cumulated seropositivity (were considered positive those samples that were positive at least two of the four applied methods).

Because the test evaluation indicated the commercial ID.vet and IFAT had the highest Jouden index, as well as because of economic reasons, IFAT can be chosen for processing pig serum samples for epidemiological studies. IFAT has the advantage that the results are obtained quick and the interpretation of the results is facile, without special equipment required. The serial dilution of serum samples is helpful for establishing the titer of *T. gondii* antibodies.

The purpose of this study was to determine the seroprevalence of *T. gondii* antibodies from indoor pig serum samples from Romania, evaluated by IFAT, MAT and ELISA.

Many serologic tests have been used for the detection of IgG anti-*T. gondii* antibodies: indirect hemagglutination test (IHA), latex agglutination test (LAT), Sabin–Feldman Die Test (DT), complement fixation (CF), IFAT, MAT and ELISA. IHA and LAT have poor resolution and sensitivity when compared with IFAT, ELISA and MAT (5, 12, 13). Additionally, IHA and LAT were considered insensitive to detect *T. gondii* infection in pigs (2).

IFAT and MAT may detect antibodies that appear at early stage of infection against components of the membrane of tachyzoites (12, 14). Differently, indirect ELISA’s are capable of detecting antibodies later in the time-course of infection (12). Moreover, ELISA and IFAT are able to distinguish IgG from IgM, while MAT does not. However, the production of IgM in pigs have short live, and therefore, this may not be a good antibody to evaluate the time of infection in this species (16).

In this study were evaluated four serological methods, two “in house” (IFAT and MAT) and two commercially ELISA (ID.vet and Safe-Path) to establish which is more sensitive and specific for anti-*T. gondii* antibodies detected in pig serum samples.
The best agreement was obtained between ID.vet ELISA and IFAT ($k = 0.89$) followed by Safe-Path ELISA and IFAT ($k = 0.7$). The high sensitivity was obtained by IFAT ($Se = 97.4$) and the high specificity by ID.vet ELISA ($Sp = 100$). Our results suggest that IFAT would be useful for detecting anti-\textit{T. gondii} antibodies in pig serum samples. This result are sustained by Garcia et al. (2006) which, comparing IFAT and MAT with ELISA, found a best agreement between IFAT and ELISA ($k = 0.88$). Because of the advantages it presents (results are obtained quickly, facile interpretation of the results, economical reasons), IFAT can be chosen for processing pigs serum samples for epidemiological studies.

Acknowledgments

This study was supported by the Ministry of Education, Research and Innovation from Romania through Executive Unit for Financing Higher Education, Research, Development and Innovation from Romania, Grant PNII PC 51-013/2007.

References


SEASON DISTRIBUTION OF GASTROINTESTINAL HELMINTHS OF GOATS IN SOUTH-EAST SERBIA

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Summary
The study about gastrointestinal helminthes of goats at south-east parts of Serbia was performed in period March 2010 to January 2011. Coprological and post-mortem examination revealed the following gastrointestinal helminthes prevalence: We revealed same parasite species: Teladorsagia (Ostertagia) circumcincta (95.23%), Ostertagia trifurcata (91.53%), Ostertagia ostertagi (23.33%), Trichostrongylus axei (100%), T.colubriformis (89.57%), Nematodirus spathiger (100%), N. filicoliis (43.31%), Haemonchus contortus (88.95%), Marshallagia marshalli (23.77%), Skrjabinema caprae (13.28%), Bunostomum trigonocephalum (13.28%), Chabertia ovina (64.14%), Oesophagostomum venulosum (28.39%), Cooperia curticei (60.52%) and C.punctata (5.26%).

The dynamics of the first occurrence of established species of gastrointestinal strongilida was as follows: In March: Teladorsagia (Ostertagia) circumcincta, Ostertagia ostertagi, Trichostrongylus axei, Trichostrongylus colubriformis, Nematodirus filicoliis and Nematodirus spathiger; In April: Ostertagia trifurcata; In May: Ostertagia occidentalis, Trichostrongylus axei, Cooperia punctata, Bunostomum trigonocephalum and Chabertia ovina; In June: Skrjabinema caprae; In July: Haemonchus contortus, Cooperia curticei and Oesophagostomum venulosum; In November: Marshallagia marshalli. Species in the genus Ostertagia, Trichostrongylus and Nematodirus were present after the first appearance of those present during the entire study period. Haemonchus contortus is ordered in animals during the warmer and Marshallagia marshalli during the colder period of the year. Species in the genus Cooperia and Oesophagostomum, Bunostomum were often present in lambs sacrificed during all the monitoring period. Species in the genus Cooperia and Oesophagostomum, Bunostomum were often present in kids sacrificed during the monitoring period.

Key words: goats, helminths, season distribution

Goats population in Serbia had a drastic decrease after II WW, but in last decade were stared to anew increase of its population espacialy at mautntain areas. Today, goats play important role in south-east part of Serbia in providing animal protein for diet especially for those people who live in the vilage. They are usually kept under extensive conditions and graze or brows on any land that is not being cultivated. Pasture breeding make possible contact within sheep and eggs, larvae stages and intermediate host of parasites. Those induce that there are no
one goat or sheep without parasites. Examination of goats parasitoses were only sporadically performed and we had only a few paper about it (5,6,16,17,21,22,23,24). From these reason in mind, during aplication of project we started with examination of parasitic fauna of goats at warious pats of Serbia. At first, we started at Stara planina

**Material and methods**

The study about season distribution of gastrointestinal helminthes of goats at south-east parts of Serbia was started in March 2010 and finished in January 2011. During study we collected fecal samples at monthly intervals. A total of 910 fecal samples were analyzed using standard coprological techniques. A total of 67 we were analyzed by post-mortem examination. Total differential worm counts were performed on all the alimentary tract and lungs using the technique described by Pavlović and Andelić-Buzadžić (18).

Determination of adult helminthes and eggs of parasites were done by keys given by Euzeby (3).

**Results and discussion**

The faecal samples were obtained from a different source all together as they were collected from flocks in the field, and the results support the other findings. These counts were also of value in providing some information on the egg rise. The number of guts and lungs examined in this survey thought small in number, but in combination with results of coprological examination, samples appeared to represent the population adequately.

We revealed same parasite species: *Teladorsagia* (Ostertagia) circumcincta (95,23%), *Ostertagia trifurcata* (91,53%), *O.ostertagi* (23,33%), *Ostertagia occidentalis* (21,37%), *Trichostrongylus axei* (100%), *T.colubriformis* (89,57%), *Nematodirus spathiger* (100%), *N. filicollis* (43,31%), *Haemonchus contortus* (88,95%), *Marshallagia marshalli* (23,77%), *Skrjabinema caprae* (13,28%), *Bunostomum trigonocephalum* (13,28%), *Chabertia ovina* (64,14%), *Oesophagostomum venulosum* (28,39%), *Cooperia curticei* (60,52%) and *C.punctata* (5,26%).

The epidemiology of the helminth parasitic diseases therefore depends on factors such as the infection pressure in the environment and the susceptibility of the host species (or individual). Furthermore, the availability of large numbers of susceptible definitive and intermediate hosts will increase the parasites’ ability to reproduce and result in high parasite abundance (11).

The life cycles of all found helminths species are direct, requiring no intermediate hosts, which applies to all of the economically important strongylid parasites of small ruminants. In these cycles, adult female parasites in the GI tract produce eggs that are passed out with the faeces of the animal. Development
occurs within the faecal mass, the eggs embryonate and hatch into first-stage larvae (L1), which in turn moult into second-stage larvae (L2), shedding their protective cuticle in the process (1). During this time the larvae feed on bacteria. The L2 moult into third-stage larvae (L3), but retain the cuticle from the previous moult. The L3 constitute the infective stage, and these migrate onto surrounding vegetation where they become available for ingestion by grazing sheep and goats. The development, survival and transmission of the free-living stages of nematode parasites are influenced by micro-climatic factors within the faecal pellets and herbage. These include sunlight, temperature, rainfall, humidity and soil moisture. At climate condition which are present in examined areas The dynamics of the first occurrence of established species of gastro-intestinal strongilida was as follows:

- In March: Teladorsagia (Ostertagia) circumcincta, Ostertagia ostertagi, Trichostrongylus colubriformis, Nematodirus filicollis and Nematodirus spathiger;
- In April: Ostertagia trifurcata
- In May: Ostertagia occidentalis, Trichostrongylus axei, Cooperia punctata, Bunostomum trigonocephalum i Chabertia ovina;
- In June: Skrjabinema capra;
- In July: Haemonchus contortus, Cooperia curticei and Oesophagostomum venulosum;
- In November: Marshallagia marshalli

Species in the genus Ostertagia, Trichostrongylus and Nematodirus were present after the first appearance of those present during the entire study period.

Haemonchus contortus is ordered in animals during the warmer and Marshallagia marshalli during the colder period of the year. Species in the genus Cooperia, and Oesophagostomum. Bunostomum were often present in lambs sacrificed during all the monitoring period. Species in the genus Cooperia, and Oesophagostomum. Bunostomum were often present in kids sacrificed during the monitoring period.

At the beginning of our research, conducted in March, the real extent of gastrointestinal infections strongilidae was 83.33%, after which he soon reached a level of 100% in the same way and moved to the end of follow-up period.

Extensity of infection established genera gastrointestinal strongilidae was different. The distribution of parasites of the genera Ostertagia, Trichostrongylus and Nematodirus was reached during the monitoring period almost the maximum level.

The distribution of species within the established genera also varied. Within the genus Ostertagia most abundant were dominated by Teladorsagia (Ostertagia) circumcincta and O.trifurcata. Prevalence of infection with Ostertagia ostertagi and Ostertagia occidentalis was higher during the colder periods of the year.

Among the species of the genus Trichostrongylus was the most prevalent Trichostrongylus colubriformis. Extensity of infection with Trichostrongylus axei and T.vitrinus varied, without any regularity. Extensity of infection with Nematodirus
filicollis and N. spathiiger demonstrated a tendency to increase and leveled off at the highest level of the whole study period. When we compared our results to the similar survey at mountain area of Serbia that have been done at Šara Mountain (11, 13), Homolje (10), and at East Serbia (5, 6, 8), we were concluded that season distribution were different with our results. During those examination at East Serbia (including Šara Mountain) strongillidae of genera Haemonchus, Ostertagia, and Nematodirus was most abundant at spring and summer and strongillidae of genera Trichostrongylus and Marshallagia during outumn and winter period (21). Similarly season distribution of helminths we established et small ruminants breed at Belgrade area (19, 20).

Similar results were obtained during the studies in neighboring countries. In Macedonia Iliev (6) and later Georgievski et al. (5) occured Trichostrongylus specis at spring months, and Marshallagia marshalli, Chabertia ovina and Oesophagostomum species only at winter period. Similarly results were established in Montenegro by Karanfilovski (9), in Bulgaria by Denev and Kostov (2) and in Romania by Ardelaeanu et al. (1).

The obtain results confirm that the seasonal distribution of gastrointestinal helminths of small ruminants depends on the microclimate of environmental conditions.

Acknowledgments

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IMMUNOENZIMATIC ASSAY TO DETECT GIARDIA SPP. IN CALVES FROM WESTERN AND SOUTH-WESTERN ROMANIA

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Summary

This study was conducted to determine the prevalence of Giardia spp. infection in calves of different age from western and south-western of Romania (Arad, Timiş, Caraş-Severin, Meheştin County), and to analyze the potential risk factors that support this infection. Faecal specimens were collected from 621 calves from birth to age of nine months. The examination of the samples was performed using Giardiasis Ag Cypress Diagnostics ELISA kit. The prevalence of the Giardia spp. in calves was 38.48% (239/621). Of the four counties studied Giardia spp. was most prevalent in Meheştin 45.74%. Overall, pre-weaned calves (eight weeks of age) exhibited the highest prevalence (39.63%), followed by post-weaned calves (two-six months of age) (36.84%) and heifers (six-nine months of age) (18.18%). The most receptive breed to infestation with Giardia spp. was found to be Charolaise breed 70% (7/10) followed by Limousin breed with prevalence 42.50% (34/80).

Key words: Calves, Giardia spp., prevalence.

Giardia is a protozoan parasite found worldwide. In one study Geurden et al., in 2006 shows the clinical importance of a natural Giardia infection in calves between the age of one and six months, since all farms reported problems of chronic and often mucous diarrhea, reduced weight gain and ill thrift (8).

A number of North American and European studies have shown the Giardia spp. infection to be highly prevalent in dairy calves with infection rates as high as 100% in some herds, and have demonstrated an association between parasite infection and resultant diarrhea and significant production losses (16, 28).

The aim of the present study was to determine the prevalence of the Giardia spp. infection, in calves from western and south-western Romania and to analyze the potential risk factors that support this infection (gender, age and breed).

Materials and methods

A total of 621 faecal samples were collected and examined between October 2009 and March 2012, from four counties: Timiş, Arad, Caraş Severin and Meheştin. The age of calves included in the study ranged from four days to nine
143 faecal samples were collected from Arad County, 134 from Timiș County, 250 from Caraș-Severin County and 94 from Mehedinți County (table 1).

For an accurate epidemiological evaluation studies were carried out in different areas of the counties mentioned above and records were kept in order to help in data interpretation.

<table>
<thead>
<tr>
<th>County</th>
<th>Locality</th>
<th>No. of samples examined in this study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arad</td>
<td>Curtici</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>Pecica</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td>Sofronea</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Utviniş</td>
<td>15</td>
</tr>
<tr>
<td>Timiș</td>
<td>Tormac</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td>Pișchia</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>Săcălaz</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Km 6 Farm</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Cenei</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Topolovățu Mare</td>
<td>13</td>
</tr>
<tr>
<td>Caraș-Severin</td>
<td>Răcășdia</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>Grădinari</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td>Vrani</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td>Berliște</td>
<td>86</td>
</tr>
<tr>
<td>Mehedinți</td>
<td>Cârșu</td>
<td>94</td>
</tr>
</tbody>
</table>

Breeds of calves in the study were: Montbeliarde (no=62), Charolaise (no=10), Fleckvech (no=4), Limousin (no=80), BBB crossbreed (no=3), Charolaise crossbreed (no=4), Limousin crossbreed (no=58), Montbeliarde crossbreed (no=34), BGXRH crossbreed (no=13), Romanian Black Spotted (no=210) and Romanian Spotted (no=143).

Feces have been individually collected directly from the rectum, in coproculture containers, from calves and then transported to the Parasitology laboratory of the Faculty of Veterinary Medicine Timisoara, being stored at a temperature of 4°C and processed in a short time.

The examination of the samples was accomplished using ELISA immunodiagnostic kits in the discipline of Parasitology and Parasitic Diseases. Some samples were processed by rapid tests H&R Crypto-Giardia (Vegal Pharmaceuticals, Spain). Antigens have been detected in faeces.
Results and discussions

The results obtained from analysis of faeces with Tecan microplate reader at 450 nm wave length are presented in table 2. The color intensity is directly proportional of the stool antigen quantity.

<table>
<thead>
<tr>
<th>Parasitism with Giardia spp. in calves in western and south-western Romania</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>County</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Arad</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Timiș</td>
</tr>
<tr>
<td></td>
</tr>
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<td></td>
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<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Caraș-Severin</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Mehedinți</td>
</tr>
<tr>
<td><strong>Total</strong></td>
</tr>
</tbody>
</table>

In Arad County 143 faecal samples were examined by ELISA out of which: 21 were positive for Giardia spp. in Curtici, 20 were positives with Giardia spp. in Pecica, five were positive for Giardia spp in Șofronea, seven samples were identified with Giardia spp. in Utviniș respectively.

In Timiș County 134 faecal samples was examined of which 52 (38.80%) samples were found positives for Giardia spp. All the samples were analyzed using rapid tests H&R Crypto-Giardia.

In Tormac samples were collected during two different seasons. In autumn of 2009, 28 samples were collected and in spring of 2010 were 29 samples collected. In autumn 12 was positive with Giardia spp. and in spring 13 were positive with total 25 (43.85%) in this locality. Other localities in which infection with Giardia spp. were identified from Timiș County have been: seven samples from Pîșchia, six samples in the micro-farm from Săcălaș, five samples from km 6 farm, two samples from Cenei households, seven from Topolovățu Mare respectively.
In Caraş-Severin County 250 faecal samples were examined by ELISA out of which: 26 were positive for *Giardia* spp. in Râcăşdia, 12 were positive in Grădinari, 23 were positive in Vrani and 30 were positive in Berlişte, respectively.

In Mehedinţi County 94 fecal samples were collected, out of which 43 were positive for *Giardia* spp. (45.74%) by ELISA. Overall, the analysis of faeces in the western and south-western Romania, 240 were positive for *Giardia* spp. (38.64%). The highest prevalence of *Giardia* spp. was recorded in Mehedinţi County, 45.74% by ELISA. Calves positive for *Giardia* spp. presented yellowish diarrhea with a nasty smell.

In table 3 are presented the infection with *Giardia* spp. according to the age.

<table>
<thead>
<tr>
<th>County</th>
<th>Locality</th>
<th>No. of samples</th>
<th><em>Giardia</em> spp. No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arad</td>
<td>&gt;1 week</td>
<td>8</td>
<td>2</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>1-3 weeks</td>
<td>42</td>
<td>12</td>
<td>28.57</td>
</tr>
<tr>
<td></td>
<td>3-5 weeks</td>
<td>28</td>
<td>9</td>
<td>32.14</td>
</tr>
<tr>
<td></td>
<td>5-8 weeks</td>
<td>25</td>
<td>16</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>2-6 months</td>
<td>29</td>
<td>11</td>
<td>37.93</td>
</tr>
<tr>
<td></td>
<td>6-9 months</td>
<td>11</td>
<td>2</td>
<td>18.18</td>
</tr>
<tr>
<td>Timiş</td>
<td>&gt;1 week</td>
<td>11</td>
<td>2</td>
<td>18.18</td>
</tr>
<tr>
<td></td>
<td>1-3 weeks</td>
<td>27</td>
<td>15</td>
<td>55.55</td>
</tr>
<tr>
<td></td>
<td>3-5 weeks</td>
<td>64</td>
<td>26</td>
<td>40.62</td>
</tr>
<tr>
<td></td>
<td>5-8 weeks</td>
<td>6</td>
<td>2</td>
<td>33.33</td>
</tr>
<tr>
<td></td>
<td>2-6 months</td>
<td>26</td>
<td>8</td>
<td>30.76</td>
</tr>
<tr>
<td>Caraş-Severin</td>
<td>&gt;1 week</td>
<td>9</td>
<td>3</td>
<td>33.33</td>
</tr>
<tr>
<td></td>
<td>1-3 weeks</td>
<td>23</td>
<td>9</td>
<td>39.13</td>
</tr>
<tr>
<td></td>
<td>3-5 weeks</td>
<td>99</td>
<td>41</td>
<td>41.41</td>
</tr>
<tr>
<td></td>
<td>5-8 weeks</td>
<td>57</td>
<td>20</td>
<td>35.08</td>
</tr>
<tr>
<td></td>
<td>2-6 months</td>
<td>62</td>
<td>18</td>
<td>29.03</td>
</tr>
<tr>
<td>Mehedinţi</td>
<td>1-3 weeks</td>
<td>7</td>
<td>1</td>
<td>14.28</td>
</tr>
<tr>
<td></td>
<td>3-5 weeks</td>
<td>26</td>
<td>15</td>
<td>57.69</td>
</tr>
<tr>
<td></td>
<td>5-8 weeks</td>
<td>7</td>
<td>1</td>
<td>14.28</td>
</tr>
<tr>
<td></td>
<td>2-6 month</td>
<td>54</td>
<td>26</td>
<td>48.14</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>621</strong></td>
<td></td>
<td><strong>239 (38.48)</strong></td>
<td></td>
</tr>
</tbody>
</table>

Measuring the results found in the three groups divided by age, the prevalence of the *Giardia* spp. infection was, the animals aged eight weeks (pre-weaned) have the highest number of infected cases 174/439 (39.63%), followed by
the group aged between two and six months (post-weaned) 63/171 (36.84%) and
by heifers group age between six and nine months with a prevalence of 18.18% (2/11) (table 4).

Table 4

<table>
<thead>
<tr>
<th>Age</th>
<th>No. of samples</th>
<th>Giardia spp. No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-weaned</td>
<td>439</td>
<td>174</td>
<td>39.63</td>
</tr>
<tr>
<td>Post-weaned</td>
<td>171</td>
<td>63</td>
<td>36.84</td>
</tr>
<tr>
<td>Heifers</td>
<td>11</td>
<td>2</td>
<td>18.18</td>
</tr>
<tr>
<td>Total</td>
<td>621</td>
<td>239</td>
<td>38.48</td>
</tr>
</tbody>
</table>

Regarding the gender factor, 318 samples belonged to males and 303 belonged to females. 119 samples derived from males and 120 samples derived from the females were positive for Giardia spp.

Positive faecal samples according to breed were as follows: 24 for Montbeliarde breed; seven for Charolaise breed, 34 for Limousin breed, 18 for Limousin crossbreed, eight for Montbeliarde crossbreed, three for (BGXRH) crossbreed, 87 for Romanian Black Spotted breed and 58 for Romanian Spotted breed (table 5).

Table 5

<table>
<thead>
<tr>
<th>Breed</th>
<th>No. of samples</th>
<th>Giardia spp. No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Montbeliarde</td>
<td>62</td>
<td>24</td>
<td>38.70</td>
</tr>
<tr>
<td>Charolaise</td>
<td>10</td>
<td>7</td>
<td>70</td>
</tr>
<tr>
<td>Fleckvech</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Limousin</td>
<td>80</td>
<td>34</td>
<td>42.50</td>
</tr>
<tr>
<td>BBB crossbreed</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Charolaise crossbreed</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Limousin crossbreed</td>
<td>58</td>
<td>18</td>
<td>31.03</td>
</tr>
<tr>
<td>Montbeliarde crossbreed</td>
<td>34</td>
<td>8</td>
<td>23.52</td>
</tr>
<tr>
<td>BGXRH crossbreed</td>
<td>13</td>
<td>3</td>
<td>23.07</td>
</tr>
<tr>
<td>Romanian Black Spotted</td>
<td>210</td>
<td>87</td>
<td>41.42</td>
</tr>
<tr>
<td>Romanian Spotted</td>
<td>143</td>
<td>58</td>
<td>40.55</td>
</tr>
<tr>
<td>Total</td>
<td>621</td>
<td>239</td>
<td>38.48</td>
</tr>
</tbody>
</table>
The highest prevalence of *Giardia* spp. infection was 70% (7/10) in Charolaise breed followed by Limousin breed with a prevalence of 42.50% (34/80).

For calves, the infection rate varied significantly in different studies, being of 17.4% to 31.3% in Belgium (7, 9, 10), 43.6% in Denmark (20, 22), 38.0% in Germany (18), 30.0% in Italy (3), 49.0% in Norway (13), 2.2% to 14.0% in Poland (2), 9.0% in Portugal (24), 26.6% to 30.1% in Spain (4, 5), 8.7% to 57.0% in Canada (1, 6, 11, 23, 27, 36), 19.1% to 52.0% in the United States (14, 31, 32, 33, 34, 35), 3.7% in Taiwan (15), 10.2% in Vietnam (9, 10), 58.0% in Australia (27), 4.5% to 40.6% in New Zealand (17, 21, 25, 29), and 8.0% to 10.0% in Uganda (12, 19). The age of the calves and housing, feeding, and management practices probably contributed to the different infection rates observed. The infection rates were also different when various detection methods were used (26). Several longitudinal studies revealed cumulative infection rates of 73 to 100% in calves (18, 30, 31, 36).

**Conclusions**

The prevalence of parasitism with *Giardia* spp. in calves in western and south-western Romania was of 38.48% (239/621).

The most prevalent 45.74% (43/94) *Giardia* spp. infection was found in Mehedinți County.

Regarding the age of the calves involved in the study, the highest prevalence was identified in calves from three day to eight weeks of age (pre-weaned), meaning 39.63% (174/439).

The breed and gender did not represent any considerable risk factors.

**References**

5. Castro-Hermida, J.A., Carro-Corral, C., Gonzalez-Warleta, M., Mezo, M., Prevalence and intensity of infection of *Cryptosporidium* spp. and *Giardia*


IDENTIFICATION OF AN PROLIFERATIVE ENTEROPATHY OUTBREAK AT SLAUGHTER AGE OF PIGS

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Summary

Macroscopic examination of the gastrointestinal masses coming from slaughter pigs, revealed the present of intestinal adenomatosis in 86 cases of the 97 probes studied. Histopathology revealed the epithelial hyperplasia accompanied by moderate loss of goblet cells, proliferation of immature enterocytes due to increased mitotic index and the presence of histio-neutrophilic infiltrate in the intestinal mucosa. Using immunohistochemistry (IHC), the causative agent of this enteritis was revealed in enterocytes and macrophages of the small intestine with or without gross lesions.

Keywords: diarrhea, porcine intestinal adenomatosis, intracellular pathogens.

Porcine proliferative enteropathy is a common diarrheal disease of young breeding and growing-finishing pigs characterized by hyperplasia and inflammation of the ileum and colon.

The disease, commonly called ileitis, occurs worldwide and produces variable clinical manifestations including a chronic form, called porcine intestinal adenomatosis (PIA), and an acute form, called proliferative hemorrhagic enteropathy (PHE). The etiology is an intracellular gram-negative small rod-shaped bacterium named Lawsonia intracellularis (4, 5).

Studies showed a high prevalence of this disease. Between 30 -50% swine herds coming from different types of production systems and in all parts of the world are infected. It was the most common disease problem in grower-finisher pigs reported in the 2000 National Animal Health Monitoring System survey, occurring on more than a third of all sites and reported on 75% of large sites (2).

The main impact of the disease has been due to increased use of antibiotics, high percentage of mortality in acute form and low average daily gains in chronic forms.

Materials and methods

Gastrointestinal masses coming from a pig slaughterhouse with closed circuit, were subjected to morphological examination, histopathological and immunohistochemical techniques. It was examined a number of 97 samples.
Even it was observed intestinal adenomatosis lesions, originating swine farm has not a history of disease. During the growing and fattening, most pigs hadn’t symptoms of enteritis. Rare cases evolved were cure after treatment for 7 days with chlortetracycline and tiamulin and mortality wasn’t registered. Moreover, their average daily gains were 580-750g.

Protocol for slides stained (H&E) (6):
- Samples were paraffined, after keeping them for 48 hours in 10% formaldehyde.
- The paraffin block was cut at 5 µm.
- Dewaxing involved 3 successive baths of toluene, 3-5 minutes each one.
- Dehydration in decrease concentration of alcohol (absolutely, 96° and 80°) was followed by hydration with distilled water for one minute.
- Stained with H&E (Haematoxylin solution - 1 min., Eosin solution – 1 min.)
- Before clearing with toluene (1 bath) and mounting, the samples were dehydrated with increase concentration of alcohol (80°, 90°, absolutely).

Immunohistochemical technique (IHC) involves antigenic exposure and immunostaining. Initially samples are subject to inclusion in paraffin technique, sectioning, dewaxing and rehydrating, according to the above mentioned protocol. Antigenic exposure was performed by exposing of dewaxed and rehydrated sections to heat, into a sodium citrate solution at pH 6, for 30 minutes. To block endogenous peroxidase was used hydrogen peroxide 3%. Immunostaining involved use of work system NovoLink Max Polymer Detection System (Novocastra, Newcastle UponTyne, UK). All steps were made using DakoCytomation Autostainer immunohistochemistry machine. Chromogen used consisted of 3.3 - diaminobenzidine and for counter-stain was applied Lille haematoxylin. All samples were double staining using alcian blue coloration.

Samples were processed and interpreted at Victor Babeș University of Medicine and Pharmacy, Histology discipline. Microscopic evaluation was realized using Nikon Eclipse E 600 microscope and images were captured with LUCIA G system.

Results and discussions

Macroscopic examination of gastrointestinal masses from slaughter pigs, revealed enteritis in 86 (89.69%) cases of the 97 studied. There was no observed hemorrhagic proliferative enteropathy or necrotic enteritis, only intestinal adenomatosis. It pointed cerebriform aspects of the ileum, jejunum and sometimes of the first third of colon (Fig. 1). Serous edema was accompanied by increased proliferation of the mucosa, the presence of folds and narrowing of the intestinal lumen. There were no reported bleeding, ulcerative or necrotic lesions.

Measurements to determine the extent of the pathological process revealed an average of 132 cm, with minimum of 7 cm and maximum 260 cm. Have been reported 3 cases (3.48%) of regional ileitis, 73 cases (84.88%) with
lesions of the ileum and distal third of jejunum and in 10 (11.62%) samples the pathological process was extent till the first third of colon.

Fig. 1. Cerebriform aspects of the small intestine

Histopathological lesions were noted on the surface of the intestinal epithelium and sometimes in intestinal glands. Epithelial hyperplasia was accompanied by moderate loss of goblet cells, proliferation of immature enterocytes due to increased mitotic index and the presence of histio-neutrophilic infiltrate in the intestinal mucosa. In some cases the only histopathological lesion observed was lympho-histiocytic infiltrate, being present even in the muscular tunic of the small intestine.

IHC revealed the presence of bacterial antigen in enterocytes and in macrophages. Of the 86 samples with macroscopic lesions, bacterial antigen was revealed in 82 specimens. Negative results obtained for the 4 samples (4.65%) subject to IHC in terms of visible lesions, may be due to end-stage of intestinal adenomatosis (3) or may confirm the hypothesis of the absence of bacterial antigens after 35 postinfectious days (1).

Immunohistochemical examination of the 11 samples without characteristic lesions of proliferative enteritis allow us to the identify 3 (27.27%) immunoreactive pigs.

Studies showed that 28 postinfectious days, macroscopic and microscopic lesions resolve (1). This could be the explanation of the lack of intestinal adenomatosis lesions in our study. Failure of identify the antigen in all samples strengthens the hypothesis that after 3 weeks of infection the bacterial antigen can lake, but this doesn’t confirm the lack of anterior infection. In the end, maybe this is the reason of low prevalence of proliferative enteropathy reported from authors at the slaughter age of pigs (5).
Fig. 2. a. Lymphocytic infiltrate, moderate depletion of goblet cells, proliferation of enterocytes (Col. H&E, 20X10); b. Presence of bacterial antigen in enterocytes and in macrophages (IHC double staining with alcian blue, 10X10)

Conclusions

Macroscopic, microscopic and immunohistochemical examination of gastrointestinal masses, allow us to identify an proliferative enteropathy at slaughter age of pigs.

Histopathological lesions at the slaughter age of pigs, had a moderate character, observed intestinal mucosal epithelial hyperplasia with or without moderate loss of goblet cells and the constant presence of lymphocytic infiltrate.

The absence of macroscopic lesion didn’t confirm the hypothesis of negative immunological response to the *L.intracellularis* antigen. Similarly, negative results obtained for samples subject to IHC in terms of visible lesions, can be expected.

References

DETECTION OF PH INDICATOR PAPER OF BOVINE MASTITIS IN COMPARISON WITH CALIFORNIA MASTITIS

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Summary

In the present study bovine mastitis was investigated using California mastitis Test (CMT) and pH indicator paper bromothymol blue in Khartoum State, Sudan. Ten dairy farms were selected randomly, from each farm 10 cows were selected among the total of hundred cows. Six farms suited in Khartoum North, two farms in each Khartoum and Omdurman locations. A simple bromothymol blue indicator was evaluated for farms diagnosis of bowline mastitis. The test required highly absorbent bolting paper impregnated with four sports of bromothymol blue indicator color scores 1 to 4 for quarter foremilk's increased with somatic cell count and pH, although variability within each color score was large. Sensitivity of the bromothymol blue test ranged from 51 to 56% and specificity from 89 to 90% for most reference criteria used to classify normal and abnormal milk. In this study the results revealed that pH indicator bromothymol blue was more accurate to detect subclinical mastitis than California mastitis Test. Results of previous study showed that 73% the general infected farms which considered very huge in dairies farms that due of lack of biosecurity and prevention to control the disease. Examine of bacteriology was performed to samples. The sensitiveness of bacteria to antibiotics was found that most common bacteria Staphylococcus, Streptococcus and E. coli were sensitive to erythromycin, and penicillin cephoxitin.

Key words: BMT, CMT, Zoonotic disease, Bromothymol blue

Bovine mastitis is the most costly disease to the dairy industry worldwide, with losses estimated at 1.4 billion dollars per year in United State (15). This disease characterized by an in-crease somatic cells especially leukocytes in the milk and by pathological changes in the mammary gland tissue (13) causes economic losses, but also hold the risk for the transmission of zoonotic disease like tuberculosis, brucellosis leptospirosis and streptococcal sore throat to human beings (2). Several methods were applied to detect bovine mastitis from which California Mastitis test (CMT) was used for a quality measurement of somatic cells counting milk, a screening test for subclinical mastitis that can be used easily at cow-side (9). The use of CMT to identify infected quarter has been extensively validated in cows that were not in early lactation. The California is rapid accurate cow-side to help determine somatic cell count in specific cow.

A simple bromothymol blue indicator test was evaluated for farm diagnosis of mastitis, the test required highly absorbent paper impregnated with four sport of
reagent of bromothymol blue. Indicator color scores (1 to 4) for quarter for milk increased with somatic cell count and pH although variability within each color score. PH-indicator technique in positive reaction the spot was charge from yellow to green or bluish green. PH indicator paper can detect rapidly streptococcus mastitis (11). The milk of healthy cows changes the indicator color to yellowish green. The test can be used by dairy producers to screen herds with relatively high incidence of mastitis or used in combination with cow cell counts to local abnormal quarters. However, the disease can be control by pasteurization technique, but a variety of bacteria still contribute illness and disease outbreak (6). The milk from an infected animal is the main source of pathogenic bacteria (6).

Bovine mastitis is caused mainly by certain Staphylococcus and Streptococcus species include Staphylococcus aurous, staph, chromo genes staph-epidemidis, staph scinaltans and Streptococcus agalictiae sprepto dysgalactiae and Streptococcus bevis (5). One of the common types of chronic mastitis is caused by the bacteria Staphylococcus aurous which is often significant, subclinical and extremely difficult to control by treatment alone (12). The most commonly used antibiotics on conventional dairy farms were penicillin, cephalosporin and tetracyclines. For mastitis penicillin, ampicilin and tetracycline are suitable for treatment of Bovine mastitis. The main purpose of this study was to find the efficiently of California mastitis test to detected subclinical mastitis n comparison with pH indicator paper, and to define the incidence of mastitis in Khartoum state. Confirm the etiological agent of bovine mastitis and sensitive test of bacteria to antibiotic.

Materials and methods

The study was conducted in Khartoum State included three provinces Khartoum, Khartoum North and Omdurman. Ten Farms were randomly selected, and ten cows were selected from each farm. Experimental animals were cross breed Friesian, local breed Butana, and Kenana breeds. The test applied to detected bovine mastitis California mastitis test (CMT), which required a plastic paddle having reagent was dispensed in plastic bottle provided with a fine nozzle. Equal volume milk and reagent was put in each cup and grittily rotated by movement of the paddle in horizontal plane, the reaction was observed immediately. The California mastitis test (CMT) with rapid mastitis test reagent was done on 2 ml of test milk mixed with 2 ml of reagent. Results of California mastitis test (CMT) were scored on scale 1 to 4, corresponding to increasing viscosity of the milk reagent mixture. Score was assessed as a normal quarter and scores 2, 3 and 4 as abnormal quarters.

pH indicator paper bromothymol blue was made to determine mastitis (Mana fractured by Kruse company in Denmark). The test was applied by adding one drop of milk on yellow sport and to observe the change in color, within 1 to 2 min of sampling the bromothymol test the color of each indicator sport was scored
on a scale 1 to 4 according to color standards. Score 1 (pale green) was assessed as a normal quarter and scores 3.3 and 4 (increasing from moderate green to dark blue green) were assessed as abnormal quarters (3).

Milk samples were collected under strict aseptic condition as stated by Barrow and Felltham (3). Samples of milk were immediately frozen in ice and simplified for bacteriological examination within 24 hours. Tool of bacteria logical exam in examination sterile Bijou bottles after cleaning the outer surface of the adder and teal with potassium permanganate and with cotton wool soaked in 70% alcohol. The love milk was stripped off and about 5 ml of milk were drawn in sterile Bijou bottle. All samples collected were immediately placed on ice in thermo flask after collection. The two media used in culture were blood agar and Macconkey agar. After culturing the plates were incubated at 37°C purification was achieved by further sub culturing on nutrient agar incubated at 37°C for 24 hours. After purification the plates were examined for cultural characteristic and biochemical reactions according to standard key (3). After isolation and identification of bacteria the sensitivity test was applied by multi discs of different antibiotics, put the culture incubation for 24-48 hours at 37°C aerobically. The effectiveness read by diameter of growth inhibition around different antibiotics multi discs.

Results and discussions

The California mastitis test was applied to detected mastitis in ten farms in Khartoum State. Result obtain for this test showed that the lower farms were infected by mastitis reputed at Sudan University farm (Kuku). Percentage of injection was 3% which can be easily controlled. The private sector ① farm and University of Khartoum the moderate percentage of infected farm by mastitis recorded 10-16%. The highly infected farm by mastitis was reported in Kafoury farm the precipitate record at 48%, that due to bad management and lack of biosecurity to prevention and control. The paper bromothymol blue was tested in ten farms the results revealed that the lower infected farm by mastitis registered in Sudan university Kuku the percentage recorded 23%, the moderate infected farm by mastitis was reported in Judiciary and private section the percentage 66-60%. Kafoury farm also held the highly infected mastitis which reported at 88-87 which consider bad indicator for biosecurity in this farm and Khartoum University farm the problem due lack of udder hygiene and efficient isolation infected cows form others and not applicable the treatment in early stage. Incidence of mastitis in Khartoum university farm reasons of the disease which presents sub clinical mastitis this study consist with Batavani et al. (3). Accordingly, from results obtain Sudan University farms Kuku, Shambat recorded lower occurrences of bovine mastitis in California mastitis test and pH indicator paper that due of respect the biosecurity program. The pH indicator paper characterized than other test of mastitis can easy detect sub clinical mastitis. Technique is more accurate sensitive, easy and rapid. It
can be done in the field at the time of the milking collection which consistent with the results of the previous work and reports (8, 15).

California Mastitis Test (CMT) is easily carried and considered as known and largely used to detect mastitis. The result of the test is not affected by external factors. The last diagnostic test takes about 15 seconds which is longer than pH indicator paper test. This test needs skilled personnel to perform it. Laboratory examination of pathogenic slides revealed that the pathogens were *Streptococcus*, *Staphylococcus* and *E. coli* and they are more sensitive antibiotics like erythromycin, penicillin and cephoxitin. The results are in agreement with the findings of (10). The study revealed that the farms with cross breeds cows showed the highest incidence of mastitis. It seems inevitable to rise cross breed cows for high milk production. It was observed that in farms of manual milking the spread of mastitis was higher.

Previous studies showed that heredity has influence on mastitis incidence in special in Holstein Friesian. The mastitis incidence varied between 9-22% which is great if compared with other reproduction characteristics. The studies also showed that the correlation between heredity and environmental interaction in the highest mastitis incidence. The environmental impact varies from difference environments in the research conducted by (1).

### Table 1

**California mastitis test for the infected Animals**

<table>
<thead>
<tr>
<th>Name of Farms</th>
<th>No. of cows tested</th>
<th>No. of teats test</th>
<th>Total no. of teats</th>
<th>Morbidity rate %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Private sect ①</td>
<td>10</td>
<td>5</td>
<td>33</td>
<td>38</td>
</tr>
<tr>
<td>Private sect ②</td>
<td>10</td>
<td>5</td>
<td>35</td>
<td>40</td>
</tr>
<tr>
<td>Sudan university Kuku</td>
<td>10</td>
<td>1</td>
<td>3</td>
<td>40</td>
</tr>
<tr>
<td>Judiciary Farm</td>
<td>10</td>
<td>8</td>
<td>31</td>
<td>39</td>
</tr>
<tr>
<td>Sudan university Shambat</td>
<td>10</td>
<td>1</td>
<td>38</td>
<td>39</td>
</tr>
<tr>
<td>University of Khartoum</td>
<td>10</td>
<td>4</td>
<td>35</td>
<td>39</td>
</tr>
<tr>
<td>Private sect ③</td>
<td>10</td>
<td>6</td>
<td>32</td>
<td>38</td>
</tr>
<tr>
<td>Private sect ④</td>
<td>10</td>
<td>4</td>
<td>36</td>
<td>40</td>
</tr>
<tr>
<td>Kafoury farm</td>
<td>10</td>
<td>19</td>
<td>21</td>
<td>40</td>
</tr>
<tr>
<td>Private sect ⑤</td>
<td>10</td>
<td>8</td>
<td>32</td>
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</table>
Table 2

<table>
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<tr>
<th>Name of Farms</th>
<th>No. of cows test</th>
<th>No. of teats test</th>
<th>Total No. of Teats</th>
<th>Morbidity rate %</th>
</tr>
</thead>
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<tr>
<td>Private sect ①</td>
<td>10</td>
<td>25</td>
<td>38</td>
<td>66%</td>
</tr>
<tr>
<td>Sudan university Kuku</td>
<td>10</td>
<td>9</td>
<td>40</td>
<td>23%</td>
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<td>Judiciary Farm</td>
<td>10</td>
<td>24</td>
<td>39</td>
<td>62%</td>
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<td>10</td>
<td>39</td>
<td>26%</td>
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<tr>
<td>University of Khartoum</td>
<td>10</td>
<td>34</td>
<td>39</td>
<td>87%</td>
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<tr>
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<td>10</td>
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<td>24</td>
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<tr>
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<td>40</td>
<td>88%</td>
</tr>
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<td>26</td>
<td>40</td>
<td>65%</td>
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</tbody>
</table>
Fig. 2. pH indicator paper test for the infected animals

Table 3

<table>
<thead>
<tr>
<th>Type of antibiotics</th>
<th>Types of bacteria</th>
<th>Staphylococcus</th>
<th>Streptococcus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythromycin (ERY)</td>
<td>Sensitive</td>
<td>More sensitive</td>
<td></td>
</tr>
<tr>
<td>Penicillin (Pen)</td>
<td>Less sensitive</td>
<td>Sensitive</td>
<td></td>
</tr>
<tr>
<td>Kanamycin (KAN)</td>
<td>Sensitive</td>
<td>Resistance</td>
<td></td>
</tr>
<tr>
<td>Furoxoludone (FUR)</td>
<td>Sensitive</td>
<td>Resistance</td>
<td></td>
</tr>
<tr>
<td>Cephalosporin (CEP)</td>
<td>Sensitive</td>
<td>Resistance</td>
<td></td>
</tr>
<tr>
<td>Oxacillin (OXA)</td>
<td>More sensitive</td>
<td>Resistance</td>
<td></td>
</tr>
<tr>
<td>Tetracyclin (TET)</td>
<td>Sensitive</td>
<td>Sensitive</td>
<td></td>
</tr>
<tr>
<td>Nalidix Acid (NAL)</td>
<td>Less sensitive</td>
<td>Resistance</td>
<td></td>
</tr>
</tbody>
</table>

Conclusions

Continuous monitoring of mastitis and its management is essential for the well-being dairy herd. This can be achieved through the detection of inflammation at its early stages and, subsequently, the detection and treatment of the mastitis infection. Traditional and well-established tests include SCCs and culture-based methods. We recommend the development of novel analytical platforms incorporating enzymatic assays, immunoassays, biosensors, and nucleic acid tests are progressively replacing the more conventional methods. Also, with advances in proteomics and genomics, new biomarkers are being discovered, allowing the disease to be detected at earlier stages. This will lead to assays with higher sensitivity, which can provide additional quantitative information on the level of inflammation ‘on-site’ and ‘on-line’ and which are also faster and less expensive. Furthermore, recent advances in microfluidics will facilitate the development of improved technologies that could subsequently be incorporated into automatic monitoring systems and portable assays for sensitive and rapid detection of mastitis.
References

THE USE OF THE DECISION TREE FOR ACARI CONTROL IN CHICKEN EGG LAYER FARMS

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Summary

On a 60 000 chicken egg layer farm, bred in vertical battery, acari occurred, and, in order to find the optimal substance and concentration for acari control, the decision tree method was used. Four Emerite products were used: 24 % imidacloprid and 3.2% deltamethrin, Pertex (0.3 % permethrin 93% and 0.3 % piperonyl butoxide 80%), Bombex (1.8 % abamectin) and formic acid. For each product work solutions of 0.5, 1, 1.5 and 2% were prepared, which were put in contact with the acari in combination with feed. As working methods direct contact, pulverizing and sprinkling were used. After determining the optimal concentration for each product, they were pulverized in the shelter. After each application acari samples were taken from the feed in order to establish the product’s effect. After finding out the optimal concentration, the use of the decision tree allowed, the calculation of the best price, based on the product usage.

Key words: pest control, economic efficiency.

The decision tree may be a useful tool for the veterinary physician, allowing them to make pertinent decisions from a technical, epidemiological and economical point of view. The proper understanding of the circumstances characterizing a chicken egg layer farm starts immediately after making the decision to populate the farm because the place, from which animals are to be brought, is highly important from epidemiological point of view. Should the farm be populated with supply/purchase animals, the animals shall comply with the same requirements.

Materials and methods

On September 10th 2012, a poultry farm with a raising capacity of 60,000 heads received pullets with ages between 14 and 20 weeks from five locations with different sanitary-veterinary conditions. The population was made separately and different sections were assigned depending on the farms from which the pullets were brought. However, all sections were fostered by the same poultry shelter. This poultry shelter consists of vertical batteries, seven
levels of 120 cages on a row, having therefore an overall length of 130m, 8m height and 1.2m width.

Feeding, watering, disposal of dejections, collection of eggs and ventilation are made mechanically, based on previous programming. After starting to lay eggs, the hens adjusted relatively satisfactory to both the schedule and the tendency to maintain and observe the laying eggs-routine. On February 14th 2013, the presence of acari, on the fifth row of the battery, was noted.

A plan of cohabitation with acari has been elaborated, knowing very well the fact that complete elimination of the acari from the coop was impossible. To solve this problem by finding an optimal substance and concentration for the acari control, the decision tree method was used. Four products have been studied:

- **Emerite** products (24 % imidocloprid and 3.2% deltamethrin),
- Pertex (0.3 % permethrin 93% and 0.3 % piperonyl butoxide 80%),
- Bombex (1.8 % abamectin),
- Formic acid.

**Results and discussions**

Work solutions of 0.5; 1; 1.5 and 2% were prepared from every product stated above; in laboratory, these solutions were put in contact with the acari in combination with feed. As working method direct contact, pulverizing and sprinkling methods were used. After determining the optimal concentration for every such product, the substances were pulverized in the shelter and after every such application acari samples were taken from the feed to determine the effects of the product.

The decision tree is drawn by placing the problem as the tree stem, the solutions that have been put forth as the tree branches, the values of the solutions and the pecuniary quantification thereof as the tree leaves (table. 1).

Examining the values emphasized by table 1, we find that all substances that have been subject to analysis had relevant effects and the differences between these products were determined by the contact time and the concentration thereof. When making the final decision, the price of the products was also taken into account.

**Emerite** was efficient at a 1.5% concentration after 12 hours, as contact time, and the price was of RON 120.00; therefore, we may conclude that it failed to comply with the qualification requirements.

**Pertex** was efficient starting from 1% concentration after 60 minutes of contact, and the price amounted to RON 140.00; therefore, we may conclude that it has been qualified for application.

**Bombex** was efficient starting from 1% concentration after 12 hours of contact with acari; the price thereof was of RON 110.00; therefore, we may conclude that it failed to comply with the qualification requirements.
The tree leaves

Table 1

<table>
<thead>
<tr>
<th>Tree stem</th>
<th>Tree branches</th>
<th>Tree leaves</th>
<th>Time/ effect</th>
<th>Value</th>
<th>Price (lei)/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.5%</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.0</td>
</tr>
<tr>
<td>Emerite</td>
<td>1.0%</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>1.5%</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>0.5%</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.0</td>
</tr>
<tr>
<td>Pertex</td>
<td>1.0%</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>1.5%</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>0.5%</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.0</td>
</tr>
<tr>
<td>Bombex</td>
<td>1.0%</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>1.5%</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>0.5%</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>0.35</td>
</tr>
<tr>
<td>Formic acid</td>
<td>1.0%</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>0.35</td>
</tr>
<tr>
<td></td>
<td>1.5%</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>0.30</td>
</tr>
</tbody>
</table>

The Formic Acid was efficient starting from 0.5% concentration, with a contact time of 60 minutes and with the price amounting to RON 90.00; we may conclude that it has been qualified for application.

Conclusions

To decide what substance and concentration are to be used, the concentration-based efficiency, the contact time, the time required for the degradation in the environment and the price were taken into account. The first position was occupied by the formic acid the second place was occupied by the Pertex which was followed by the Emerite and Bombex.

References

EFFECTS OF SHORT TERM ADDITION OF CLINOPTILOLITE TO COLOSTRUM ON SOME BIOCHEMICAL PARAMETERS IN NEWBORN CALVES

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Summary

The objective of this research was to determine if the short term supplementation of clinoptilolite in colostrum influence some blood biochemical parameters, indicators of kidney and liver function, in newborn calves. Our research included 20 newborn calves that were divided into two groups: control group (C, received colostrum without clinoptilolite) and experimental group (E, received colostrum with 0.5% clinoptilolite added in the first three colostrum meals). Blood samples were collected from jugular vein in vacutainer tubes from all calves prior to colostrum intake and after that, at 24 and 48 after calving. Samples were analyzed for α-amylase, total bilirubin, creatinine, uric acid, urea, glucose, cholesterol and triglycerides. The dynamics of biochemical parameters measured in the first 48h after birth showed that morphfunctional changes of the newborn organism for adapting to extrauterine environment were normal, without any influence of clinoptilolite.

Key words: calf, clinoptilolite, biochemical parameters

Due to the synepitheliochorial placenta encountered in ruminants, calves are born with no immunoglobulins in the blood stream. Acquiring passive immunity is achieved exclusively through ingestion and absorption of adequate amounts of colostral immunoglobulins (18), that’s way the colostral period represents an important moment for the newborn calf. Starting of a good protection against neonatal diseases depends on how this period is managed.

The incidence of passive immunity failure is still high in young ruminants and is caused especially by not respecting the most important factors: time of providing the first colostrum feeding after birth, quality and quantity of colostrum (4,20). Research studies showed that reducing the failure passive transfer (FPT) by improving the absorption of Ig can be done with clinoptilolite, a natural zeolite (3, 17).

Fratrić et al. (2005) reported up to 40% higher IgG concentration during the first 48 hours of life in newborn calves that received 5g/l clinoptilolite in 0.75l/1.5l colostrum at 12 h intervals than in control groups (3). This results and those of Šamanc & al. (16) and Sadeghi & al. (15) highlights the idea that the resistance of
calves to infections in their first weeks of life is in accordance with the level of serum γ globulins concentrations and that enhancement of passive immunity in newborn calves leads to other positive effects like reducing the incidence of diarrhea and increasing average weight.

Zeolites are crystalline, hydrated aluminosilicates of alkali and alkaline earth cations, having three-dimensional structures capable of trapping molecules of proper dimensions for further release or elimination. Biological effects of zeolites make use of one or more of their properties, such as ion exchange capacity, adsorption and related molecular sieve (10,11).

Although this natural product does not cross the intestinal barrier, we know from other studies (8,19) and from our previous research (21), that zeolites have also good results on some mineral parameters, like Ca and Fe.

From this reason, the aim of this research was to investigate the evolution of other biochemical parameters (creatinine, total bilirubin, α-amylase, uric acid, cholesterol, triglycerides, glucose) in the first 48h postpartum, in calves that received clinoptilolite added in colostrum in comparison with calves that received only mother's colostrum.

Materials and methods

The study was carried out on 20 newborn Holstein calves in a farm with 540 dairy cows, in Western part of Romania. Calves were separated from the dams within 10-20 min after parturition and were not allowed to nurse; they were weighed and transferred to individual pens. Calves were fed nipple bottle two times daily, at 12 hours interval, with their mothers’ colostrum (3L). Colostrum feeding was continued for 5 days. After that colostrum was replaced with herd milk, together with concentrate; water was available ad libitum.

The calves were assigned in the two groups based on birth order. The first group of calves were control group (C, n=10) and the other group were experimental group (E, n=10). Calves from experimental group (E) received colostrum with 0.5% clinoptilolite, starting immediately after parturition, at 12 and 24 hours. The blood samples were collected from jugular vein in vacutainer tubes prior to colostrum intake, at 24 and 48 hours after birth.

Serum was obtained after centrifugation the samples at 3000 g for 5 minutes and stored at -20°C until analysis.

The commercial product used in experiment was Min-a-Zel S, from PATENTKOMERG, Belgrad, Serbia. The chemical composition of Min-a-Zel S is mostly SiO₂ (64.21%) and Al₂O₃ (11.48%) and was determined at ITNMS (Institute for the Application of Nuclear Energy), Serbia.

The activities of α-amylase (CNPG3 liquid method), total bilirubin (Jendrassik-Grof method), creatinine (Jaffe kinetic method without deproteinization), uric acid (enzymatic colorimetric method), urea (urease UV liquid method), glucose (GOD-PAP method), cholesterol (CHOD-PAP method) and
triglycerides (GPA-PAP method) in serum samples were determined with commercially available kits (Hospitex Diagnostics, Italy) using biochemical automatically analyzer (EOS Bravo Forte, Hospitex, Italy). HD calibrator serum, HD normal serum and QC serum (Hospitex, Italy) was used for controlling measurement accuracy.

The results obtained are expressed as means ± SE. One way analysis of variance (ANOVA) was used to test for statistically differences (p<0.05) between the groups of calves and inside the group.

Results and discussions

The results obtained are represented in table 1, where we can observed the evolution of the parameters determined in the first 48h after birth.

Table 1

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group</th>
<th>At parturition</th>
<th>24</th>
<th>48</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total bilirubin (mg/dl)</td>
<td>C</td>
<td>0.79±0.05</td>
<td>0.93±0.15</td>
<td>0.78±0.22</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>0.72±0.10</td>
<td>0.92±0.08</td>
<td>0.94±0.12</td>
</tr>
<tr>
<td>Creatinin (mg/dl)</td>
<td>C</td>
<td>2.04±0.16</td>
<td>1.30±0.11</td>
<td>1.09±0.19</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>2.49±0.22</td>
<td>1.35±0.08</td>
<td>1.25±0.05</td>
</tr>
<tr>
<td>Uric acid (mg/dl)</td>
<td>C</td>
<td>1.12±0.13</td>
<td>1.14±0.09</td>
<td>1.50±0.24</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>1.28±0.07</td>
<td>1.06±0.17</td>
<td>1.22±0.14</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>C</td>
<td>28.06±9.05</td>
<td>25.44±4.78</td>
<td>27.78±7.24</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>31.07±5.55</td>
<td>24.48±4.44</td>
<td>23.35±6.07</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>C</td>
<td>53.75±9.32</td>
<td>147.17±13.98</td>
<td>131.52±12.77</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>53.36±10.53</td>
<td>145.43±24.95</td>
<td>131.10±23.06</td>
</tr>
<tr>
<td>α amilase (U/l)</td>
<td>C</td>
<td>23.29±2.76</td>
<td>25.05±2.21</td>
<td>24.94±2.22</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>22.64±2.95</td>
<td>30.20±4.26</td>
<td>26.08±3.23</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>C</td>
<td>20.26±2.28</td>
<td>41.39±2.83</td>
<td>70.47±3.47</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>29.05±3.79</td>
<td>44.41±5.34</td>
<td>71.92±4.74</td>
</tr>
<tr>
<td>Triglicerides (mg/dl)</td>
<td>C</td>
<td>20.75±3.24</td>
<td>28.32±3.40</td>
<td>59.14±7.54</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>23.91±5.4</td>
<td>25.94±2.68</td>
<td>37.55±4.90</td>
</tr>
</tbody>
</table>

Significant differences (p<0.05) inside the group: *vs parturition; **vs 24h
Significant differences (p<0.05) between the groups: *vs 48h

In the first 24h of life total bilirubin, glucose, α-amilase, cholesterol and tryglicerides increased, and creatinin, urea and uric acid decreased in both groups, with no differences between the groups. At 48h after birth the evolution of parameters was similiar to the one from 24h, except for urea (E/C: -15.94%, p<0.64) and tryglicerides (E/C: -36.50%, p<0.02).
Increased of total bilirubin in the 48h after birth observed in all calves is physiological due to the process of fetal erytrocite destruction that adjusts in the first 14 days of life (12). Urea and creatinine level were maximum immediately after birth and after that decreased at 48h postpartum, beeing close to normal values found in adult cow (0.6-1.8 mg/dl creatinine and 7.8-25 mg/dl urea nitrogen)(5). These decreases may reflect the continuous increase in glomerular filtration rate (7). A slice difference between groups at 48h (E/C: -15.94%, p<0.64) in urea values could indicates a reduction of protein catabolism in E group, important for the newborn calf immunity, since most proteins are gammaglobulins with an important role in local defense, in the gut, but also in overall defense of the body. Increased total bilirubin and decreased creatinine and urea values in the first week after parturition were observed also by other researchers (2,7). According to other studies, increase urea levels, along with total proteins and GGT values in newborn calves, can be used as an indicator of passive transfer, as a result of a high protein diet (colostrum)(13).

Glucose increased significantly in both group after consuming colostrum (C 0h/24h: p<0.001, 0h/48h: p<0.001, and E group 0h/24h: p<0.002, 0h/48h: p<0.006) with no differences between groups. Compared with adult reference value (42-75 mg%)(5), these values are 2-3 times higher. This could be due to colostrum consumption and/or increased levels of corticosteroids during parturition, according to Mohri et al. (2007)(9).

Activity of α amilase increased after birth, but with no influence of group. Compared with adult reference value (41-98 UI)(5), newborn calves secretes amylase at a significantly lower rate. This lower rate of synthesis is also influenced by the high-protein diet (colostrum)(6).

Triglycerides increased significantly at 48h after birth in both groups (C group 0/48h: p<0.0004; E group 0/48h: p<0.04), higher in C group (C/E: p<0.02 at 48h), also cholesterol increased significantly (C group 0/24h: p<0.0001; 0/48h: p<0.0001; E group 0/48h: p<0.001) after consuming colostrum, similar to other reports (7,14). This increased underline the importance of colostrum for ensuring energy suport of the newborns, necessary especially in termoreglation and neoglucogenesis. It is well know that colostrum is twice or more higher in fat content than normal milk (1).

The short time surveillance of the dynamics of biochemical parameters reflects the important role of colostrum for the newborn calves and also the intensity of the metabolism that take place, necessary to adapt calves to the new environment.

Conclusions

Biochemical values observed in the first 48h in the newborn calves, values that were physiological for the neonatal period, and the close values between C and E groups obtained from this experiment highlights the conclusion that short-
time clinoptilolite supplementation did not affect hepatic and renal function of newborn calves.

Acknowledgments

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References


A SURVEY (OR PRESENCES, DINAMICS, PREVALENCES) OF CULICOIDES (DIPTERA: CERATOPOGONIDAE) IN GORJ COUNTY, ROMANIA, PRELIMINARY RESULT OF ENTOMOLOGICAL SURVEILLANCE FOR BLUETONGUE

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Summary

Entomological investigation of the presence of Culicoides species in Gorj County, Romania, was conducted in 2012. The aim of the research was to establish the presence of the main vector of bluetongue virus in a goat farm. We proceed for weekly capture of vectors (one/week) with a stable trap, for one year, in a target location and systematic vector capture with mobile trap.

During the investigation, a total of 54 samples, 6788 insects (30 samples with other insects and 8 with Culicoides) were collected and two species was identified, Culicoides obsoletus and Culicoides pulicaris. The presences of Culicoides biting midges in study area represent a risk factor for the development of Blue tongue in susceptible animals to disease.

Key words: Culicoides, survey, goats

Culicoides is a genus of biting midges in the family Ceratopogonidae found around the world including 39 fossil records from Baltic amber, and have been classified among 39 subgenera (2; 9). The family Ceratopogonidae (Diptera) currently includes 6056 species of which 1322 species belong to the genus Culicoides (1, 14).

Most Culicoides are hematophagous insects and several species are known to be vectors of various diseases and wide range of viruses, bacteria, protozoa, nematodes and helminths which can affect domestic and wild animals and humans (3, 4, 6).

At least three orbiviruses (Reoviridae) namely African horse sickness (AHSV), bluetongue (BTV) and epizootic haemorrhagic disease (EHDV), transmitted by these midges, cause diseases of such international significance that they have been classified as notifiable to the Office International des Epizooties (OIE) (7, 11, 12).

The study of Culicoides distribution, activity and taxonomy is therefore of considerable importance for veterinary medicine.
The aim of the present study was to establish the presence of the main vector of bluetongue virus in a goat farm, and to determine the diversity, distribution and dynamics of *Culicoides* species in Gorj County from Romania.

**Materials and methods**

**Study area**

The study was conducted in Sâmârineşti village situated in the south-eastern part of the Gorj county. Sâmârineşti Village is bordered on the north by Slivileşti, Văgiuleştii east of the village, south Motru River, west of town Horastă - City suburb Motru and limit boundary between Gorj and Mehedinti. Community consists of nine villages: Sâmârineşti, Boca Bazavan, Valley Church, Glade Valley, Duculeşti, Țirioi, Broad and Little Valley.

The county is located in the southwestern part of Romania, on the river Jiu. Relief of county is varied and can be divided into three physical-geographical units. These are mountains, Subcarpathians Getic between rivers and Olteţ Motru, southern hills that stretch along Getic Plateau.

The climate is temperate continental with Mediterranean influences moderate. Due landscape configuration, the climate is varied according to relief. Annual average temperatures increase from north to south. The dominant winds are the north.

Gorj County is bounded on the north by the parallel of 45° 58’north, passing near the village Tantareni. Parallel 45° crosses the county in the south of his residence (13).

![Map of Gorj County](https://maps.google.com/) (15)

Fig. 1. Collection site where midges of the subgenus *Culicoides* were trapped (modified after https://maps.google.com/) (15)

Geographical coordinates of goat farm in village Samarineşti, Valley Village Church are: 23033422 longitude, 447622451 latitude and 302 m altitude.
Sampling and determination of insects

Biting midges of the genus Culicoides were trapped, during the period from January to December 2012, around goat’s farm from Sămărinești village, Gorj County, Romania. We proceed for weekly capture of vectors (one/week) with a stabile trap, for one year, in a target location and systematic vector capture with mobile trap. Midges were collected by means of light traps (CDC Ondesteport – TehSys - blacklight UV 4 W tube). Traps were placed outside the enclosures where the livestock were confined. These traps were switched on at dusk and off at dawn the following day. Collection bags were removed in the morning and taken to the laboratory to kill the insects by freezing them at -20°C.

Culicoides midges were initially sorted according to their distinctive wing patterns under a stereomicroscope (Motic SMZ 140 FBGG with video extension) using taxonomic keys.

Results and discussions

Captured individuals were first categorized according to wing morphology identifying five species of the subgenus Culicoides, some of them with high morphological variability associated. During the investigation, a total of 54 samples, 6788 insects (30 samples with other insects and 8 with Culicoides) were collected and two species was identified, C. obsoletus and C. pulicaris.

During the vector activity of 2012 year, 27 samples were collected of which 19 samples with other insects (3064) and 8 samples with 3660 insects in which 7 samples with C. obsoletus (22 insects) and 4 samples with C. Pulicaris (7 insects).

During without vector periods of 2012, 27 samples were collected of which 16 samples without insects, and 11 samples with other insects (64 insects).

The common characters of Pulicaris complex were the presence of a dark spot in the cubital wing cell, the absence of a pale spot at the end of vein M3+4 and the presence of an hourglassshaped third costal spot (9).

C. obsoletus is a species capable of sustaining BTV replication (10). It is well-known that many factors influence the vectorial capacity, but climatic factors play a very important role in periods of seasonal activity, as well as in the size of vector population. Activity of Culicoides species has not been recorded below 13°C and above 35°C (5, 8).

The obsoletus group includes the following species: C. abchazicus, C. chiopterus, C. dewulfi, C. montanus, C. obsoletus, C. scoticus. The pulicaris group includes the following species: C. almeidae, C. halophilus (= C. newsteadi), C. muscicola, C. newsteadi, C. pulicaris and C. punctatus (14).

Culicoides obsoletus was the most numerous species followed by C. pulicaris and other species of insects. This species have a real importance in bluetongue virus transmission.
Conclusions

Two species of culicoides with potential sanitary-veterinary implications were identified during investigations: *Culicoides obsoletus* and *C. pulicaris*.

The presence of Culicoides biting midges in study area represent a risk factor for the development of Blue tongue in susceptible animals to disease.

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