Pathological syndromes caused by avian hepatitis E virus (aHEV) have been described under different names, depending on the country in which they were observed. The most commonly used terms are big liver and spleen disease (BLS), hepatitis-splenomegaly syndrome (HSS) (1), necrotic, haemorrhagic, hepatomegalic hepatitis, or hepatitis-liver haemorrhage syndrome (1, 6, 8, 11, 22). In Poland, the first description of a clinical case of BLS was presented in 2011 at the 1st Veterinary Practice Congress in Łódź (31), although field practitioners had already been observing this syndrome for a few years, at least from 2007 (33).

**Taxonomy**

Hepatitis E virus (HEV) was initially assigned to the Caliciviridae family, but this was later changed when it became apparent that HEV and caliciviruses differ in many aspects (3). It was proposed that a new taxonomic unit named Heviridae (hepatitis E and related viruses) be created that would consist of hepatitis E virus and HEV-like viruses (9). Finally, in 2004, HEV was classified as a member of the Hepeviridae family (11).

Since then, the taxonomy of this family has been changed several times as new data emerged. Initially, it was proposed that the Hepevirus genus be replaced by two separate genera: Orthohepevirus and Avihepevirus (20, 21). In light of newly isolated virus strains, another change in taxonomy was necessary, and in 2014 the current taxonomic structure was established (Tab. 1). According to this classification, the Hepeviridae family is divided into two genera: Orthohepevirus and Piscihepevirus. Avian hepatitis E virus belongs to the Orthohepevirus genus and the Orthohepevirus B species (26). Emerging new research results suggest that the taxonomy of these pathogens will undergo modifications. Among these new data, new virus strains not yet assigned to any group were discovered in wild birds in 2015 and 2016. The virus strain occurring in the little egret (Egretta garzetta garzetta) had the most similar structure to that of avian strains occurring mainly in poultry, while strains occurring in birds of prey, such as the common kestrel (Falco tinnunculus) and the red-footed falcon (Falco vespertinus), had a structure similar to that of the Orthohepevirus C species (occurring in rats and ferrets), which may be associated with the diet of these birds (preying on voles, shrews and mice), and of the Orthohepevirus A species, genotype 4 (occurring in humans and pigs) (24, 25). On the other hand, a strain detected in the Himalayan vultures (Gyps himalayensis) kept in the Beijing Zoo demonstrated the highest similarity to Orthohepevirus A, genotype 3.
which is present in humans and pigs, as well as in such species as rabbit, deer and mongoose. In this case, interspecific infection was suspected, but virus multiplication in the body of birds was not confirmed (16).

It is assumed that the first clinical case of infectious hepatitis E in a human occurred as early as in 1794 in Germany, but the aetiological factor causing this disease had not been determined as HEV until 1991 (1). Before the first cases of the disease were observed in pigs (1997), the disease had been presumed to be transmitted only between humans (14).

Subsequent publications indicated that the aHEV group may be more complex, as the existence of genotypes 4 and 5 had not been confirmed (16). Since 2010, mainly thanks to novel diagnostic methods, more pathogens similar to hepatitis E virus have been discovered in rats, rabbits, ferrets, mink, foxes, bats, mice and salmonids (14).

To date, four or five genotypes of hepatitis E virus, depending on the source, have been identified. According to Huang et al. (12), the entire HEV group was divided into five genotypes, among which the genotype 5 group included isolates of avian hepatitis E virus. Subsequent publications suggested only four main genotypes, including genotypes of human and swine viruses (1). Genotypes 1 and 2 were strictly assigned to human viruses, while genotypes 3 and 4 were genotypes of viruses infecting humans, as well as pigs and other animals (19, 20, 23). In 2010, Meng (20) distinguished separate strains of poultry hepatitis E virus, but did not specifically assign them to genotype 5 (Fig. 1). Currently, it is believed that distinguishing the avian genotype, along with the human and swine genotypes, was inappropriate. Within the Orthohepevirus A genus, 7 genotypes are currently distinguished. Apart from the previously identified first four genotypes, novel genotypes 5 and 6 from wild boars and genotype 7 from camels have been isolated (35), while the genotypes of viruses isolated from birds have been grouped in the separate Orthohepevirus B genus (26).

In 2009, a division of aHEV into three different genotype groups was proposed: genotype 1 (Australian, AaHEV), genotype 2 (USA, USAaHEV) and genotype 3 (European, EaHEV). Moreover, genotype 2 was divided into two subgroups: pUSAaHEV and aavUSAaHEV. It was found that genotype 2, USAaHEV, is structurally most similar to hHEV (human hepatitis E virus) and sHEV (swine hepatitis E virus) (4, 27).

Table 1. Taxonomy of the Hepeviridae family according to Smith et al. (2014), including the aHEV genotypes proposed by Billic et al. (2009) and the recently described, but not yet assigned, species (16, 17, 24, 25, 37, 38)

| Family | Genus | Species | Main host species | Genotype |
|--------|-------|---------|------------------|----------|
| Hepeviridae | Orthohepevirus A | Human | HEV – 1 |
| | Human | HEV – 2 |
| | Human, pig, rabbit, deer, mongoose | HEV – 3 |
| | Human, pig | HEV – 4 |
| | Boar | HEV – 5 |
| | Boar | HEV – 6 |
| | Camel | HEV – 7 |
| | Hen (aHEV) also detected in: song thrush (Turdus philomelos), little owl (Athene noctua), feral pigeon (Columba livia domestica), common buzzard (Buteo buteo) | Genotype 1 Australian (AaHEV) |
| | | Genotype 2 USA (USAaHEV) pUSAaHEV aavUSAaHEV |
| | | Genotype 3 European (EaHEV) China (CaHEV) |
| | | Genotype 4 (?) |
| | | Genotype 5 (?) |
| Orthohepevirus B | Orthohepevirus C | Rat | HEV – C1 |
| | Ferret | HEV – C2 |
| | Unassigned | Bat |
| | Unassigned | Himalayan vulture |
| | Unassigned | Common kestrel |
| | Unassigned | Red-footed falcon |
| | Unassigned | Little egret |
| | Piscihepevirus A | Trout |

HEV RNA (7,200 base pair)

Fig. 1. Schematic diagram of the HEV genome structure
and 5 was suggested. Currently, the Australian genotype 1 also includes isolates originating from Korea (15), the European genotype 3 also includes isolates from China (CaHEV) (30, 38), and it is presumed that the proposed genotype 4 consists of isolates originating from Hungary and Taiwan (8, 10). The proposed genotype 5 would consist of isolates from Great Britain (18). The aHEV taxonomy is additionally complicated by observations indicating differences in the structure of isolates from Russia that, for geographical reasons, were suspected to belong to the European genotype 3, but it became apparent that they did not match the structure of any of the previously described genotypes, although they were closest to the isolates originating from China (13, 27).

On the other hand, recent reports raised doubt about the appropriateness of the classification within the Orthohepevirus B genus, as the highlighted genotypes are not very far apart from each other in terms of the genome structure. According to Smith et al. (26), the validity of the classification will be confirmed when more complete aHEV genomes are available.

In light of the currently available data, there is no doubt that avian hepatitis E virus and big liver and spleen virus (BLSV) are different strains of the same pathogen (23).

Structure of the virus

Avian hepatitis E virus is a single-strand RNA virus of 27/28-34/35 nm in size (2, 14), with positive-sense (+)ssRNA, whose genome consists of approx. 7,200 base pairs (4). It contains a short non-coding 5' terminus (27-35 nucleotides) followed by three partially overlapping open reading frames: ORF1, ORF3 and ORF2. The non-coding 3' terminus (65-74 nucleotides) has a poly(A) tail of 150-200 nucleotides (34).

The structure of the HEV genome in animal and human strains is comparable. Analysis of the avian HEV genomic sequence revealed a 50-60% similarity of the nucleotide sequence with human and swine HEVs (7).

The lack of protein envelope (as in the case of hepatitis A virus) allows the virus to be transmitted orally.

Open reading frame 1 (ORF1). ORF1 starts at the 5' terminus immediately after a short non-coding region consisting of 27 to 35 nucleotides. This fragment of the genome contains approx. 4,600 nucleotides [between 4,592 in the EF206691 genome (7) and 4,598 in the AM943646 (4) and GU954430 (40) genomes] and encodes a non-structural protein (polyprotein) of approximately 1,693 amino acids. The ORF1 polyprotein participates in the replication of virus particles and structural changes in proteins. From the N' terminus to the C' terminus, the following proteins can be observed: methyltransferase (Mt1), domain Y of unknown role (similar to rubella virus and beet necrotic yellow vein virus – BNYVV), protease similar to papain (found in alphaviruses and rubella virus), domain X of unknown role (similar to rubella virus, BNYVV, alphaviruses and some coronaviruses), a NTP-binding sequence associated with helicase activity (supports RNA unwinding required for replication and transcription) and RNA-dependent RNA polymerase (RdRP) (2, 34).

Open reading frame 2 (ORF2). The ORF2 segment, according to the data available from GenBank (NC_023425, AY535004, EF206691, AM943646, AM943647 and GU954430), consists of 1,820 nucleotides followed by a 65 nucleotide long polyadenylated terminal sequence at the 3' terminus of the ribonucleic chain. This ORF encodes the main, if not the only capsid protein. It is a glycoprotein of approximately 71-88 kDa (660 amino acids, with the saccharide component at the N terminus) containing a potential signal region (with a high concentration of arginine and lysine) for endoplasmatic reticulum. The protein is produced as a precursor which, guided by the signal sequence, is subsequently converted into a mature protein and undergoes glycosylation at three possible sites. The presence of additional saccharides is more frequent in the superficial proteins of enveloped viruses than in the capsid proteins of non-enveloped viruses (34). The knowledge of the immune properties and structure of the ORF2 proteins is very important for the production of vaccines, since the first vaccine for humans is based on HEV-neutralizing antibodies that bind capsid proteins (36). In 2017, Syed et al. (30) showed that the truncated ORF2 protein (ORF2-C) of recombinant Chinese avian hepatitis E virus can provide complete immunoprotection against CaHEV infection in chickens, whereas ORF3 protein generates only partial protection.

Open reading frame 3 (ORF3). The smallest open reading frame, ORF3, consisting of 263 nucleotides (GenBank: NC_023425, AY535004, EF206691, AM943646, AM943647 and GU954430), is located after ORF1 and overlaps 210 nucleotides (GenBank: NC_023425, AY535004, EF206691, AM943646, AM943647 and GU954430) of ORF2. It is not known precisely whether the protein encoded by ORF3 is part of the virus particle or whether it is a non-structural protein. This protein is not glycosylated, but it is phosphorylated (34). It promotes the survival and proliferation of cells. Moreover, ORF3 suppresses non-specific host response by inhibiting acute-phase reaction and promoting the secretion of immunosuppressive factors, such as alpha-1-microglobulin (2).

The size and location of each ORF slightly differ depending on the host species from which the isolate originates. Previously, a schematic diagram of the Orthohepevirus B genome was presented, taking into account the known size for aHEV, i.e. Orthohepevirus B. Interestingly, an additional open reading frame ORF4 has been detected in the genomes of viruses isolated from certain species, with the currently confirmed pres-
Molecular diagnostics

Avian HEV strains do not replicate in cell cultures. In general, there is currently no effective cell culture technique for HEV isolated from animals. According to the method of Sun et al. (29), for the isolation of aHEV, SPF chickens can be used. The presence of the pathogen should be confirmed by serological or molecular methods. In order to detect the virus in samples, polymerase chain reaction (PCR), or more specifically reverse transcription-PCR (RT-PCR), are used. aHEV RNA has been detected in bile samples and liver fragments, which confirms that the virus replicates in the liver (5).

RT-PCR is the best method of identifying avian HEV infections, but it is not known how specific it is in the detection of chicken infections in different geographical areas (11, 28). Nonetheless, Marek et al. (18) presented a paper describing a pair of primers that detected aHEV in cases originating from both Europe and Australia.

The literature presents a large variety of primers used for molecular diagnosis of avian hepatitis E virus infections in birds, which is due to the high diversity of genotypes and variability of the virus. To detect such infections, apart from the classical RT-PCR method, nested RT-PCR and real-time RT-PCR are used. Table 2 lists primers used for aHEV diagnosis arranged chronologically. Primer pairs 1, 2, 3 and 8 are used in molecular diagnostics employing RT-PCR, primer pairs 4, 5, 6 and 7 are used in nested RT-PCR, whereas primer pairs 9 and 10 are used in real-time RT-PCR, but primers designed by Troxler et al. (32) use SYBR Green dye. The primers can also be distinguished according to the part of the genome they detect: primer pairs 2, 3 and 4 detect helicase located within ORF1; primer pairs 6 and 7 detect another genomic fragment, also within ORF1; primer pairs 5 and 8 detect capsid, i.e. ORF2; whereas primer pairs 9 and 10 detect ORF3. The primers were designed at different times and based on different genotypes that occurred in a given area. Therefore, primer pair 1 was designed for and detected strains from Australia, whereas primer pairs 8 and 9 were designed for and detected strains from both Australia and Europe. In the United States, primer pairs 2, 3, 4, 5, 6 and 7 were used, and in China, primer pair 10 was used.

| #  | Name          | Nucleotide sequence (5‘–3’)                                                                 | Source    |
|----|---------------|-----------------------------------------------------------------------------------------------|-----------|
| 1  | BHE-fwd       | GCG CGG ATG TGG TTA TGCA TA                                                                 | Payne et al., 1999 |
|    | BHE-rev       | CGG CGA GCT GCA TCTCAA TA                                                                     |           |
| 2  | B1            | GCT AAG CGA CCC GCA CCA GAT                                                                   | Huang et al., 2002 |
| 3  | B2            | GTG TAG CGC AAC AAG ATG                                                                     |           |
| 4  | Helicase F    | TGG CGC ACC(T) GTT(A) TCC(T) CAC CG                                                          | Huang et al., 2002 |
|    | Helicase R    | CCT CA(G) GGA CGG TA(T) TAC TGG ACC C                                                         |           |
| 5  | AHEV F-1/SD   | TGT TAT T(C) ACC ACC CAC CAA G(A) AC ATG(T) G                                              | Sun et al., 2004 |
|    | AHEV F-2/SD   | GCC AGC GCT G(A) ATC ACC CAC(T) TAC(C) GT                                                    |           |
|    | Helic R       | CCT CA(G) GGA CGG TA(T) TAC TGG ACC C                                                         |           |
|    | Helic R-2     | GAC CCA(G) GGA(G) TCC CAC TGG                                                               |           |
| 6  | N1            | TTA CCA TTG ACT TTG AAC GGC G                                                               | Billam et al., 2005 |
|    | N2            | CGG GGC TGA TGG TCT CGA TGA                                                                  |           |
|    | N3            | GCT GTG GCA TGG ACG ATT TCC C                                                                |           |
|    | N4            | CAA TAG GTT ACC CAC GAT G                                                                    |           |
| 7  | P1            | ACA ACA TCC ACC CTA AG                                                                       | Billam et al., 2005 |
|    | P2            | ACA GTT TCA CTT CAG GCT                                                                     |           |
|    | P3            | AGA ACA ATG GTT GGC GGC G                                                                    |           |
|    | P4            | GAG GGC AGC CCA CTA AAC                                                                    |           |
| 8  | Forw1 C-BLSV  | GGT ATG GGG GTT TGG ACC ATG                                                                  | Bicil et al., 2009 |
|    | Rev1 C-BLSV   | GCT GGN CGN ARQ AGT GTC GA                                                                   |           |
| 9  | BLSV-HEV     | AAT GGG ATG GGG GTG AAA                                                                      | Troxler et al., 2011 |
|    | BLSV-HEVr    | CAT GAA GGT GGG GGT GGG G                                                                    |           |
|    | Probe ORF3-HEV| FAM-CTCA AAC GCT CCC AGC GGG A-BHQ1                                                            |           |
|    | Probe HEV-3  | FAM-CTCA AAC GGY CAG CCC AGC GGG A-BHQ1                                                        |           |
| 10 | F1            | CGT GAC AAC TCA GCC CAGTG                                                                   | Zhao et al., 2015 |
|    | R1            | GCG GTG ACA AGC TCG GCA                                                                     |           |
|    | R2            | ATG TAT CTT AGT TCG CAG TGG                                                                  |           |

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