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New developments in the diagnosis of avian influenza

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Summary

Avian influenza has become a serious concern from both veterinary and public health points of view. National and international organisations, veterinary health authorities, research institutions, diagnostic laboratories and field services make enormous efforts worldwide to detect, combat and prevent this important disease. Accordingly, the standard diagnostic protocols are being supported by a wide variety of molecular detection techniques, including improved polymerase chain reaction assays, microarray-based detection and characterisation methods, very rapid sequencing, simple pen-side tests and other on-site approaches. These recently developed 'closer to the field' methods allow rapid detection of influenza viruses and the identification of pathogenicity variants. However, in order to harmonise the diagnosis worldwide, attention has to be paid to the validation and standardisation of these technologies, to avoid erroneous interpretation of assay results, and, consequently, inappropriate epidemiological measures.

This review gives an overview of the current and potential future developments related to avian influenza diagnostics.

Keywords

Avian influenza – Isothermal amplification – Microarray – Molecular diagnosis – Pathotyping – Pen-side test – Polymerase chain reaction – Proximity ligation – Rapid sequencing – Standardisation.

Introduction

Avian influenza (AI) is a highly contagious disease of birds and is listed by the World Organisation for Animal Health (OIE) (67). The disease poses serious economic consequences because of its direct devastating effect and the stamping out and other restrictive measures incorporated into legislation aimed at controlling the disease. Because AI crosses species barriers and AI viruses (AIV) are able to infect humans, concern has increased regarding the zoonotic, and, consequently, pandemic potential of the disease. The causative agents of AI are type A influenza viruses that belong to the Orthomyxoviridae family (62). Avian influenza viruses reside in migratory water birds in nature and typically cause no clinical disease in their reservoir host (49, 50, 59).

The AIV genome contains eight single-stranded RNA segments of negative polarity that encode 11 proteins (61).

Based on the composition of the surface glycoproteins, haemagglutinin (HA) and neuraminidase (NA), influenza A viruses are divided into subtypes. Sixteen HA and nine NA subtypes have been identified, and all of the HA and NA combinations so far detected in nature have been found in birds (3, 24). Based on their distinct virulence properties, AIV are classified into low pathogenic (LPAIV) and highly pathogenic (HPAIV) strains. So far, HPAIV have been associated only with the H5 and H7 subtypes. Furthermore, because of the risk of an H5 or H7 virus of low virulence becoming virulent by mutation, all H5 and H7 AIV are notifiable (67). The H5N1 influenza A viruses have also raised serious concerns because these viruses have caused human fatalities in East Asia and Egypt (15). Moreover, the increased frequency of emergence of HPAIV H7N3 and H7N7 and the panzootic spread of H9N2 subtype viruses pose further threats of transmission to humans (2, 46). Therefore, extensive efforts have been made to improve AI diagnostics. Among the main goals of the AIV developmental work, the effective detection of AIV in its reservoirs and in diseased poultry is critical. From the point of view of outbreak control, diagnostic tests that target the virus are of great value if they can provide rapid and accurate results regarding the presence or absence of influenza virus in the specimens. These tests include virus isolation, antigen capture immunoassays, and molecular diagnostic tests such as polymerase chain reaction (PCR) and sequencing of the PCR products.

This review focuses on recent advances in molecular methods developed to improve the detection and identification of influenza viruses. However, because of the enormous amount of literature in this field (approximately 200 to 400 new publications every month, according to the Influenza Virus Resource page of the United States National Center for Biotechnology Information – http://www.ncbi.nlm.nih.gov/genomes/FLU/FLU.html), this review will concentrate on methods recommended by the reference laboratories and on selected novel trends as examples of the improved detection and identification of influenza viruses.

Current methods and present situation

The standards for AI diagnosis require the isolation (biological amplification) of the causative agent in embryonated chicken eggs from the typical samples, i.e. swabs (cloacal and oropharyngeal) or specimens from different organs (4). Isolation of the causative agent is followed by the identification of the isolate, while the haemagglutinating activity of the isolate is tested and its virulence assessed using *in vivo* or *in vitro* tests. Various methods based on reverse transcriptase PCR (RT-PCR) are now also being used in addition to these procedures, and they are expected to accelerate the diagnosis of AI and have comparable sensitivity to virus isolation, together with high specificity (60). Among these protocols, a selected set has been validated and is recommended by the European Union (EU)/OIE/Food and Agriculture Organization (FAO) reference laboratory for AI, the Veterinary Laboratories Agency (VLA) in Weybridge, United Kingdom (UK).

This laboratory uses the TaqMan® technology for the general detection of influenza A viruses, by targeting the matrix gene (57). This technique specifically detects H5 and H7 subtype viruses using a modification of the original protocol (38, 55, 57) to provide better coverage for the Eurasian isolates of these subtypes. Alternatively, a one-step conventional RT-PCR is recommended to amplify the cleavage site of H5 and H7 subtype viruses for nucleotide sequence determination (54). These protocols have been thoroughly validated by the VLA and, for instance, the Eurasian H5 subtype detection protocol was found to be so sensitive that even the biological amplification step could be omitted before subjecting a clinical sample to this test, without affecting the results (64). Nevertheless, in the case of positive results, verification by virus isolation or conventional PCR followed by nucleotide sequencing is necessary. Additionally, these protocols are tailored for H5 viruses of Eurasian origin and they should therefore be validated to reflect the evolutionary process that affects the nucleotide composition of the primer and probes in target regions. Therefore, developmental efforts are aimed at improving analytical sensitivity, and providing a reasonable tolerance of variation of the target sequence to allow direct pathotyping of H5/H7 subtype viruses.

Recent trends in the development of influenza diagnostics

Distinguishing avian influenza from Newcastle disease

When dealing with AI diagnostics, Newcastle disease (ND) is the first disease that must be included in the differential diagnosis, because infections with either HPAIV or Newcastle disease virus (NDV) result in similar clinical signs and epidemiology in poultry. Therefore, measures to control HPAI and ND are included in the same national contingency plans, as stipulated in Council Directives of the European Community (19, 20). Thus, accurate diagnosis of AI means the rapid differentiation and detection of these two diseases. Examples vary from

conventional multiplex PCR assays (17, 22) to oligonucleotide microarrays (65). Unlike the case of AIV, discriminating assays for virulence variants of NDV are scarce (e.g. see reference 66).

Improved reverse transcriptase polymerase chain reaction assays

The performance of a PCR-based diagnostic assay largely depends on the following key elements: RNA extraction, RT-PCR amplification, and the sequences of the primers and probes used (reviewed in reference 60). The following examples focus on the latter aspects:

– one-step RT-PCR giving simultaneous detection of subtype H5, H7 and H9 viruses (40)

– linear-after-the-exponential PCR (LATE-PCR), which is a new concept of nucleic acid amplification that exploits the kinetics of the reaction more profoundly than the traditional approaches (52)

– the application of Light Upon eXtension (LUX) oligonucleotide primers, which provides a simple and still accurate means of target detection that could be a good alternative to probe-based methods (6, 37).

One-step reverse transcriptase polymerase chain reaction for influenza virus detection

Three subtypes of AIV are considered to be the most important from the point of view of potential zoonoses, i.e. H5, H7, and H9 (45). Therefore, it is critical that diagnostic procedures are able to identify these subtypes in a rapid and accurate way. Monne *et al.* (40) developed and validated a specific and sensitive assay for these subtypes, which may be useful in routine laboratories for the detection and differentiation of these subtypes of great importance.

Linear-after-the-exponential polymerase chain reaction

LATE-PCR technology provides the basis for efficient, versatile and sensitive amplification assays (52; http://www.late-pcr.com). It is an advanced form of asymmetrical PCR that efficiently generates single-stranded amplicons under controlled conditions and provides a number of advantages over symmetrical PCR chemistries. These include increased levels of multiplexing, a broader temperature range over which to probe (it should be remembered that the amplicon is single stranded), and rapid DNA sequencing of the amplicon without purification. This technology also has improved sample preparation, suppressed amplification errors, and improved probe design for rapid high-resolution analysis of the amplified product, which makes multiplexing easier and allows for rapid DNA sequencing and enhanced data analysis. The combination of an easy to use portable PCR machine (see below) and the power of LATE-PCR technology provides the ability to carry out highly informative analysis on site and aids in the diagnosis of animal diseases in the field.

Light Upon eXtension polymerase chain reaction

Simplifying the set-up of a PCR assay while maintaining its characteristics is also a promising direction for development. The LUX PCR does not require oligonucleotide probe and quencher molecules; instead, like conventional PCR, it uses two primers, one of which is labelled with a fluorophore molecule. This set-up enables melting curve analysis on completion of the amplification, which provides a convenient and reliable way to confirm its specificity. The LUX PCR was tested as a broad-range assay for detection of AIV by targeting matrix gene sequences (37), and was found to be an appropriate tool for the purpose. Owing to its relatively simple set-up, LUX PCR requires less optimisation compared with other probe-based RT-PCR assays.

Isothermal amplification methods

Another way of simplifying target detection is the application of isothermal amplification protocols. Among these techniques, nucleic acid sequence-based amplification and loop-mediated isothermal amplification (LAMP) have been applied to the detection of influenza viruses (36). This is an optimised form of self-sustained sequence replication (3SR) that uses primers containing sequences for the promoter of T7 RNA polymerase, which becomes functional once the double-stranded DNA is formed after reverse transcription. These systems can be used for the detection of RNA targets, as demonstrated with influenza viruses (18). Loop-mediated isothermal amplification is a novel and expanding platform for diagnostic assay development (41, 42, 43). It offers several advantages over conventional PCR in that it is fast and cost-effective and can be carried out in a simple heat block or water bath. In addition, the end product of LAMP can be observed visually, either by examining the turbidity caused by the accumulation of magnesium pyrophosphate during the reaction, or through the addition of an intercalating fluorescent dye that binds to the amplified DNA. Therefore, LAMP may be a suitable method for diagnosing pathogens in clinical laboratories on the frontline and in poorly equipped laboratories in developing countries. The assay can be used to detect RNA simply by including a heat-stable reverse transcriptase in the isothermal reaction. For influenza viruses, H5, H7 and H9 assays have been developed, including assays for the detection of human influenza (16, 33, 34, 35). By further optimising and validating detection of the LAMP product, and making it applicable at the pen side, e.g. by using lateral flow devices, LAMP could be a suitable choice in

first-line diagnostic protocols, for instance during follow-up investigations in outbreak situations after the index case has been diagnosed.

Solid-phase microarray technologies

Microarrays and DNA chips are high-density arrays usually produced on glass or silicone (7, 11, 58). They allow the simultaneous detection of thousands of genetic elements. Furthermore, when they contain species-specific gene sequences, microarrays can be used to establish phylogenetic relationships between isolates (14, 25, 63).

The high capacity of multiplexing different microarray assays has been exploited for detecting and subtyping all possible HA and NA subtypes of AIV (29, 30). The method set up by Gyarmati *et al.* (29) uses padlock probes (PLP), which are designed such that when the 5´ and 3´ ends base pairs are next to each other on a template strand, they undergo enzymatic ligation, which circularises the probe and concatenates it to the template (8). This is followed by a two-level amplification of the circularised probe, the first level of which involves rolling circle amplification (RCA) to produce a long linear concatamer of the probe. Non-ligated probes could not be subjected to RCA. The concatamer obtained forms the template of the second PCR, which uses a fluorescently labelled primer. This set-up, i.e. initial RCA of the reacted probes, reduces the risk of amplification artefacts, thus increasing the sensitivity of the assay. This design, which allows all circularised probes to be amplified by the same primer pair and then sorted by unique microarray tags, makes the PLP system more suitable for use in highly multiplexed assays than the traditional multiplex PCR, which is limited by the number of primer pairs that can be combined in individual reaction mixtures before cross-reactivity becomes a problem (29). Another advantage of the PLP design is that it can accommodate the variability of viral genomes, especially in the case of RNA viruses. The PCR-based approaches require two conserved regions (one for each primer). In contrast, PLP needs only one recognition site on the target sequence, where the two probe arms hybridise. The PLP design can therefore provide the benefit of speed and sensitivity derived from using a nucleic acid-based method, while the amount of information is greatly increased by the high level of multiplexing.

Molecular assays for improved characterisation of avian influenza virus, including discrimination between high and low pathogenicity variants

The most widespread RT-PCR methods, which are based on TaqMan® (56, 57), provide an important but limited amount of information on the biological features of the detected influenza viruses. Because of the importance of influenza in general, and AI in particular, there are a number of different approaches gaining ground in influenza diagnostics. For example, using pyrosequencing, laboratories can determine the pathogenicity, receptor binding and glycosylation characteristics, as well as the phylogenetic traits, of H5N1 influenza viruses (48). Straightforward amplification and sequencing of the polymerase complex of influenza viruses provides further important insights into the genetic determinants of virulence (39, 51). The full-length amplification of genes followed by sequencing provides comprehensive information on influenza A viruses (10, 13), but these methods require extensive customised optimisations on an individual basis because their general application has proven to be rather difficult (39).

The Influenza Genome Sequencing Project (IGSP) was started by the United States National Institute of Allergy and Infectious Diseases in early 2004 to improve the availability of genomic sequences and related information on the influenza viruses. The IGSP began by sequencing large numbers of influenza viruses at The Institute for Genomic Research (TIGR). Before this project, only a handful of flu genomes were publicly available, whereas their number is currently over 3,000 (http://www3.niaid.nih.gov/research/resources/mscs/Influenza/). Such services are indispensable for further methodological developments and an understanding of the biology of influenza.

A single set of primers was developed by Phipps *et al.* (47) for generating PCR amplicons from a wide range of influenza A viruses, which simplifies the process of identification. Similarly, universal primers have been used to amplify the HA cleavage site region to accelerate pathotyping of H5/H7 and subtyping of non-H5/H7 subtype influenza viruses (27). These protocols greatly facilitate the recognition of new strains of influenza viruses and, with the simultaneous pathotyping capability, greatly strengthen the reliability of influenza diagnostics. A probe-based assay was developed to identify and characterise the Qinghai-like H5N1 HPAIV (32).

The differentiation of high and low pathogenic variants of AIV is routinely done by sequencing the cleavage site of the HA gene. Efforts to reduce the time required to achieve results have produced protocols based on the discrimination of the pathotypes of H5 subtype viruses using melting curve analysis (44). However, the sequence variability within the cleavage site region makes such an approach rather indeterminate for distinguishing virulence variants. Therefore, the implications of melting point differences should be evaluated with caution. For example, in cases where viruses have been isolated during an outbreak, melting point differences might have an indicative value (for the pathotype) but this is not true in

cases where viruses have originated from different outbreaks (J. Banks, unpublished), because of other possible sequence differences in the amplicons.

In addition to the accurate detection and characterisation of the HA gene, particular attention should be paid to the precise identification of NA to uncover the presence of reassortant variants. For the accurate and rather sensitive identification of N1, a capable TaqMan® minor groove binder assay was developed by Agüero *et al.* (1), and a one-step RT-PCR with a broad spectrum for influenza NAs by Alvarez *et al.* (5). Furthermore, high-resolution technologies such as microarrays can be used with good potential for the purpose (see below and reference 29).

Very rapid sequencing

Based on the highly conserved nucleotide stretches flanking the cleavage site of the HA gene of influenza viruses, the authors have developed a one-step real-time SYBR Green RT-PCR assay for the detection of a broad range of AIVs (68). The nucleotide sequencing of the amplified PCR products revealed simultaneously both the subtype and the pathotype of the AIV isolates. The general subtype identification and pathotype determination can be performed within a few hours, providing a useful alternative tool for the timely and simple characterisation of AIV isolates.

Pen-side tests

The diagnostic tests that can be accomplished at the site of an outbreak, i.e. adjacent to the livestock holding facilities, can be divided into two groups:

– simple antigen detecting tests such as lateral flow immunochromatography

– portable instruments that perform practically the same RT-PCR assays as a laboratory-based device but are compact and, in this case, faster.

Although they are easy to use, owing to their relatively low sensitivity the results obtained by lateral flow devices should always be interpreted with care and confirmed by other, more sensitive tests.

The latter group include handheld devices or mobile laboratories. In general, the immunological tests are faster while the nucleic acid-based technologies are more sensitive and specific. Several companies are producing and optimising these small, battery-powered machines, which can be easily used under field conditions, allow for complete disinfection of the equipment, require simple sample preparation, and produce rapid results without the need for specific training. An example of a portable instrument is the Handheld Advanced Nucleic Acid Analyzer which weighs less than 1 kg and is highly automated; it uses the TaqMan® RT-PCR platform and produces results within 30 minutes (31, 35). Another automated PCR-based system is the Ruggedized Advanced Pathogen Identification Device (RAPID), developed by Idaho Technology (35). This device is based on the Light Cycler PCR technology with a fluorescence detection system. An even smaller version, termed the 'RAZOR', is currently under development: reactions are performed in plastic pouches that are preloaded with freeze-dried reagents (http://www.idahotech.com/RAZOREX/index.html).

The Portable Veterinary Diagnostic Laboratory (Smiths Detection; http://www.smithsdetection.com/vet) comprises a portable briefcase-sized PCR instrument and a disposable sample preparation unit. This integrated system provides rapid on-site identification under a wide range of weather conditions for veterinarians or other workers in animal health. The operators require no technical understanding of the PCR methodology, but the equipment provides a result concerning the suspected diagnosis of a viral disease in the field. The type of sample that can be used is determined by the disease/veterinarian, and both RNA- and DNA-based pathogens can be identified with this system. Such systems typically include a global positioning system (GPS) and wireless communications so that data can be sent back to a centralised facility for surveillance or to aid in controlling disease spread during an outbreak. They use temperature-stable reagents that do not need to be kept in a fridge or freezer, and the Smith Detection system uses LATE-PCR technology (see above).

Integrated Nano-Technologies has developed a novel DNA-based biosensor that can rapidly and accurately detect and identify pathogenic organisms (21). The sensor is capable of detecting the binding of a single molecule of DNA or RNA, therefore this system does not require PCR amplification. The biosensor consists of oligonucleotide probes attached to multiple pairs of interdigitated electrodes on a microchip. Hybridisation of target DNA to the DNA capture probes that are bound to the electrodes forms a DNA bridge connecting the two electrodes. Chemical treatment of the DNA bridge coats it with metal and converts it into a conductive wire. Formation of one metallised DNA bridge reduces the electrical resistance of the sensor 1,000-fold, enabling the detection of a single molecule of DNA. Current research is focused on automation and integration of sample preparation procedures, PCR amplification, and detection into a single microchip or instrument for rapid assays with minimal user-intervention to eliminate the possibility of sample contamination and the need for highly skilled personnel to operate the system. Although these technologies are major improvements on early methods, there are some obvious limitations regarding their performance. For example,

most of the current technologies use nucleic acid-based detection systems or are exclusively immunoassay-based technologies. Therefore, they share the inherent limitations associated with these techniques in terms of sensitivity, specificity, speed, false positives, and cost. Until a fundamentally new technology is developed, process enhancements will be the best solution to these limitations. The use of orthogonal methods, such as PCR technology coupled with immunological detection systems, could provide the greatest level of confidence in the developed technologies. However, because of their prohibitive costs, the primary field of usage for these portable devices remains biodefence.

Potential new technologies

Liquid-phase microarrays using Luminex technology

The Luminex principle combines flow cytometry, fluorescent microspheres, and traditional biological chemistry into a flexible, open-architecture, multi-analyte profiling system that delivers highly sensitive, accurate and cost-effective results. Given that the Luminex can be used for detection of either proteins or nucleic acids, its use has become widespread among the different fields of potential application. These include testing for allergic diseases, immunological monitoring, identification of disease markers, studies related to pharmacogenomics and genetic testing, and detection of infectious agents.

Proximity ligation assay

The proximity ligation assay (PLA) is an approach that detects proteins via nucleic acid amplification (28). The principle is that antibodies recognising viral or bacterial surface proteins are coupled to DNA strands that can be joined by ligation when several antibodies are bound in proximity to surface proteins of individual infectious agents. This method enables the detection of target proteins by ligated DNA strands, which are then amplified by RT-PCR. Throughout the authors' studies with influenza viruses, the sensitivity of proximity ligation was higher than that of antigen enzyme-linked immunosorbent assay (AgELISA) (manuscript in preparation). Thus, PLA can efficiently aid antigen detection and be a useful tool for early diagnosis of infectious diseases; furthermore, its *in situ* version can be of great value for localising antigens, e.g. in tissue sections.

Nanotechnology

Nanotechnology biosensors have been used for pathogen detection using different approaches, e.g. detection by luminescence using quantum dots, localised surface plasmon resonance of metallic nanoparticles, enhanced fluorescence, dye immobilised nanoparticles, and Raman reporter molecule immobilised metallic nanoparticles (26). All the nanostructures used for biosensing applications have two characteristics. First, they contain certain recognition mechanisms specific to the analyte (e.g. antibodies or enzymes). Second, they are able to generate a distinguishing signal from the analyte, which can be generated by the nanostructures themselves or produced by signalling molecules immobilised or contained in the nanostructures. A potential field for the application of nanoscience in veterinary medicine is microbiological food safety testing, which is becoming a huge concern (53).

Discussion

Considering the constant threat of AI, the OIE maintains that the most effective strategy for dealing directly with this notifiable disease is early detection and early warning, rapid confirmation of disease threats, rapid and transparent notification, and rapid response (including containment, management of poultry movement, zoning and compartmentalisation, stamping out, with consideration of animal welfare, and vaccination where appropriate). Early detection and warning are key to the effective management of AI. Consequently, the OIE is actively engaged in improving the capacity of the surveillance and information systems of national Veterinary Services. The OIE has also adopted new standards for the quality and disease notification systems of national Veterinary Services and has improved its own information system to provide early and accurate epidemiological information on a worldwide basis, in particular through its Global Early Warning and Response System for Major Animal Diseases, including Zoonoses (a joint initiative developed with the World Health Organization and FAO). Rapid confirmation of suspect cases and diagnosis of AI is based on official criteria (http://www.oie.int/eng/info_ev/en_AI_prevention.htm). Chapter 2.3.4. of the OIE *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* provides the official recommendations for the diagnosis of AI, including the conventional methods for direct identification of the agents and serological tests (67).

As stated in the OIE manual and reported in several scientific articles (e.g. 4, 12), the accurate and effective diagnosis of AI is a complex task. Although a range of useful techniques is available, there is a great need for the development of new approaches, which would further increase the reliability, the robustness, the scale and the speed of diagnostic procedures. The range of molecular tools developed for the more practical, but at the same time accurate and informative, detection of influenza

viruses is steadily expanding. These methods may support or replace traditional procedures such as the determination of pathogenicity by *in vivo* tests. Conventional PCR tests are also gradually being replaced by RT-PCR protocols that are less prone to contamination and can be automated (9). There is a need for centralised facilities (where and how these are established needs to be addressed in a different forum) to perform such tests, but developments in integrated systems are likely to allow for future field testing (23).

Besides improving the sensitivity, specificity, and length of time required for the detection of viruses, much emphasis is placed on performing these tests at the site of the outbreak or suspected outbreak. Thus, relatively simple pen-side tests are being developed, for example those based on lateral chromatography or LAMP technology, which do not require sophisticated instrumentation and facilities to perform but are nevertheless accurate. Of course, making such tests widely available for livestock owners would not mean that they no longer had a duty to report notifiable animal diseases. Other versions of on-site pathogen detection tools encompass portable amplification instruments, which have so far been developed mainly for biodefence purposes.

Microarray technologies have the potential to test large numbers of pathogens simultaneously, which could make a significant contribution to the diagnostic capabilities of many laboratories. Developments in the integration of sample processing, amplification and analysis, and the eventual production of effective commercial testing devices would herald an important achievement in allowing for field testing. Advances in nanotechnology have potentially important contributions to make in this process, with the likelihood that test results could be obtained within minutes. Suitable wireless communication systems with centralised databanks and access to decision-making tools will allow for speedy therapeutic and prophylactic decision-making, a desirable achievement in any effective diagnostic programme.

The expanding possibilities in molecular biology and access to newer platforms make it tempting to explore novel ways of developing molecular diagnostic assays for infectious diseases. Once a particular protocol has been developed, evaluated and optimised, it should be followed up with proper field validation (4, 61) and constant re-evaluation.

It should also be recognised that for a change in a given procedure to occur, e.g. in the laboratory diagnosis of AI, the alternative must provide a significant advantage, which may include lower cost, increased sensitivity, improved ease of use, or improved throughput (60).

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Nouvelles avancées dans le diagnostic de l'influenza aviaire

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Résumé

L'influenza aviaire est devenue un grand sujet de préoccupation pour la santé animale et la santé publique. Partout dans le monde, les organisations nationales et internationales, les services officiels de santé animale, les institutions de recherche, les laboratoires de diagnostic et les services de terrain déploient d'immenses efforts pour détecter, combattre et prévenir cette importante maladie. De ce fait, les procédures standardisées de diagnostic s'appuient désormais sur une grande variété de techniques moléculaires de détection dont les tests perfectionnés d'amplification en chaîne par polymérase, les méthodes de détection et de caractérisation par microdamiers, les techniques de

séquençage ultra-rapides, les tests rapides de terrain et d'autres méthodes de détection de terrain. Grâce à ces méthodes « plus proches du terrain » récemment mises au point, il est plus facile de détecter rapidement les virus de l'influenza et de reconnaître les variantes pathogéniques. Toutefois, dans le but d'harmoniser le diagnostic au niveau mondial, il faut veiller à valider et à standardiser ces techniques afin d'éviter les erreurs d'interprétation des résultats des tests et les conclusions épidémiologiques inappropriées qui en découlent.
Les auteurs font le point sur les avancées actuelles et les perspectives futures dans le domaine du diagnostic de l'influenza aviaire.

Mots-clés
Amplification en chaîne par polymérase – Amplification isotherme – Diagnostic moléculaire – Essai de ligature de proximité – Influenza aviaire – Microdamier – Pathotypage – Séquençage rapide – Standardisation – Test rapide de terrain.

Nuevos adelantos en el diagnóstico de la influenza aviar

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Resumen
La influenza aviar se ha transformado en un problema grave, tanto en materia de sanidad animal, como de salud pública. En todo el mundo, organizaciones nacionales e internacionales, organismos de sanidad veterinaria, entidades de investigación, laboratorios de diagnóstico y servicios en el terreno despliegan enormes esfuerzos para detectar, combatir y prevenir esta importante enfermedad. Como corresponde, los protocolos normalizados de diagnóstico incluyen una amplia variedad de técnicas de detección molecular como, por ejemplo, pruebas mejoradas de reacción en cadena de la polimerasa, métodos de detección y caracterización mediante microalineamiento, técnicas de secuenciación muy rápida, estuches de diagnóstico de fácil utilización a pie de establo, así como otras técnicas utilizables en el terreno. Entre estas últimas se encuentran los métodos "más próximos al campo" para detectar rápidamente los virus de influenza y determinar la patogenicidad de las variantes. Pero esas técnicas deben validarse y normalizarse a la hora de armonizar el diagnóstico en todo el mundo para impedir errores de interpretación de los resultados y el consiguiente falseamiento de las medidas epidemiológicas.
En este artículo se exponen los adelantos actuales y posibles del diagnóstico de la influenza aviar.

Palabras clave
Amplificación isotérmica – Determinación de patotipos – Diagnóstico molecular – Influenza aviar – Microalineamiento – Normalización – Prueba de ligadura de proximidad – Prueba a pie de establo – Reacción en cadena de la polimerasa – Secuenciación rápida.

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