



Molecular Techniques for Rapid Diagnosis of Infectious Livestock Diseases

Salisu, I. B^{1,2*}, Amin, A. B¹, Ibrahim, A. A¹, Abdurrahman, S. L¹ and Ali, Q².

¹Department of Animal Science, Faculty of Agriculture, Federal University Dutse, PMB 7156 Dutse, Jigawa State-Nigeria.

²Centre of Excellence in Molecular Biology, University of the Punjab, Lahore, Pakistan, 87-West Canal Bank Road Lahore-53700, Pakistan.

Abstract

This review is aimed at highlighting some of the advanced technologies used currently for rapid and early diagnosis of some important animal diseases. Animal health is under continuous threat from a number of dreaded infectious diseases, which results in substantial economic loss to livestock owners. Maintenance of good animal health depends on early, precise and rapid identification of a range of diseases which are essentially infectious. Conventional diagnostic approaches have been used for infectious disease diagnosis, however, these methods are less sensitive, time consuming and labour intensive. The effective control and treatment of animal diseases requires access to diagnostic tests that are rapid, reliable and highly sensitive. The use of recent molecular technologies such as polymerase chain reaction (PCR), multiplex-PCR, DNA microarray, next generation sequencing, and improved immunoassay have now revolutionized the disease diagnostic procedures by providing precise diagnosis or detailed characterization of any pathogen or host pathogen interactions. These advanced methods are highly sensitive, rapid, specific, less tedious and helps in early disease detection. Thus, proper application of these techniques will enhance effective control and treatment of these important animal diseases.

Key words; Livestock, Infectious disease, Molecular diagnostics, Multiplex-PCR,

Introduction

Livestock industries in many parts of the world, particularly developing countries have been faced with the devastating effects of infectious diseases which upsets national and international trades (Tomley and Shirley, 2009). Although, substantial improvement has been made during the last few years in reducing the pervasiveness of animal diseases of economic importance, there is still an increasing concern over the losses associated with infectious diseases that cause decrease in production efficiency (Christy and Thirunavukkarasu, 2006). The increase in the emergence or re-emergence of infectious diseases have been evident for many decades (Gibbs, 2005). Various conventional diagnostic approaches have been used for detecting the infectious agents either through

culture or antibody detection, however, these approaches are less sensitive, expensive and time-consuming. (Shojaei *et al.*, 2015). For effective treatment and control of infectious animal diseases, diagnostic tests that are rapid, reliable and highly sensitive are required (Shyma *et al.*, 2015). Recent advances in the field of molecular biology have allowed the molecular -based diagnostic tests (i.e DNA or Protein based) of the infectious diseases, making it possible to identify the disease-causing agent(s) at a species/sub-species/type level and monitor the impact of disease control programmes (Lew-Tabor, 2009). These innovative approaches used in the field of infectious diseases have widened the scope of veterinary diagnostics and provided powerful new tools that allows for rapid and specific diagnosis of animal diseases.(Tsalik *et al.*,

salisu.ib@fud.edu.ng

+2347066006080,

+923174632425,

2018). Therefore, this review will highlight the application of advanced protein and DNA based diagnostic approaches for highly sensitive, specific and rapid pathogen identification.

Protein based diagnostic approaches

The detection of pathogens by protein-based methods is based on antibody-antigen interactions in which specific antibody will bind to its target antigen (pathogen) present in a given sample. The sensitivity and specificity of the method is directly related to the binding strength of a particular antibody to its specific antigen (Zhao *et al.*, 2014).

Immunoassays are the most commonly used protein based diagnostic test for the detection of pathogens from blood, serum and other biological fluids (Lew-Tabor, 2009). These types of assays are widely used due to their wide diagnostic time window, as antibodies for a pathogen may persist for up to a year, and this makes the assays valuable for investigating the past exposure to vector-borne pathogens. An additional advantage of this assay is the ease of sample collection and ability to detect current infections via sero-conversion (that is, the period during which a specific antibody develops and becomes detectable in the blood) (Kuleš *et al.*, 2017).

Enzyme-linked immunosorbent assay (ELISA) is one of the most widely used immunoassay for detection of various diseases of veterinary significance. This is an immunological test that involves the use of antibody/enzyme conjugate that produces a coloured reaction to detect antibodies or any infectious agent in a sample. This technique has been used widely for the detection of parasite-specific antibodies, antigens and immune complexes. Use of ELISA for the diagnosis of a number of pathogens including *Theileria* spp and *Babesia* spp has already been reported (Salih *et al.*, 2015).

Immunoassays largely depend on culture for the production of antigens. However, the use of purified antigens is limited due to inability to produce the proteins in sufficient quantity. Therefore, improved immunological tests are needed to overcome this constraint in cases where cultures are not available (Prajapati *et al.*, 2017). Recently, various recombinant or purified antigens are produced via modern molecular approaches such as gene cloning, expression of immunogens, over expression of vectors as well as peptide synthesis. These recombinant or purified antigens are widely used to improve diagnostic specificity and sensitivity (Elmahallawy *et al.*, 2014). These methods have mainly been used for the development or improvement of ELISAs and can be applied to produce highly sensitive screening (designed to detect all infected animals) and specific confirmatory assays (designed to detect only infected animals which limits the number of false-positive reactions) (Schmitt and Henderson, 2005).

Efforts in the improvement of immunoassays has also led to the development of a new approach called biosensor immunoassay. This type of assay uses a receptor (usually an antibody) for detection of the target pathogen or a disease-specific antibody and a transducer which converts biological interaction (that is, interaction of the target protein with an immobilized antibody) into a quantifiable signal based on electrochemistry, fluorimetry, interferometry, resonance and reflectometry. And the produced signal is directly proportional to the amount of antigen present in the sample (Shyma *et al.*, 2015). These types of assays have now been established for the diagnosis of bovine anaplasmosis and classical swine fever (Lew-Tabor, 2009).

DNA based diagnostic approaches

During the past two decades, the conventional techniques used for the detection of infectious

agents have transited from protein and culture-based methods to DNA based testing assays which allows for selective amplification of specific targets present in very low concentrations often below the detection limit of conventional methods to detectable levels (Gasser, 2006). The most commonly used DNA based diagnostic methods are briefly elucidated in the following sub-headings;

Polymerase chain reaction (PCR) based diagnosis

The term PCR refers to a biochemical process that copies and amplifies DNA using thermally stable enzyme called DNA polymerase (Loftis and Reeves, 2012). PCR is a molecular technique with the widest variety and application in veterinary diagnostics. The strength of this technique is due mainly to its simplicity, sensitivity, flexibility, and ability to produce millions of copies of a target deoxyribonucleic acid (DNA) (Sokolová, 2017).

Through PCR technology non-cultivable or slow-growing microorganisms such as mycobacteria, anaerobic bacteria, or viruses from tissue culture assays and animal models can easily be detected (Pfaffl *et al.*, 2009). The technique can also help in identifying the sequence of previously unknown viruses related to those already known, thereby making it easier for better understanding of the disease itself. Detection of infectious agents and the discrimination of non-pathogenic from pathogenic strains by virtue of specific genes are the main basis for PCR diagnostic applications in veterinary diagnosis (Cai *et al.*, 2014). PCR technology was first applied in veterinary diagnostics for specific genomic detection of bovine viral diarrhea virus, foot and mouth disease virus, Infectious bovine rhinotracheitis, Buffalopox, and Ephemeral Fever virus (Ridpath and Bolin, 1998). The use of PCR in veterinary diagnosis has been expanded, and it was

applied for the swift and large-scale diagnosis of avian influenza and Newcastle disease during recent outbreaks of these diseases in the USA (Schmitt and Henderson, 2005).

Reverse Transcription-PCR (RT-PCR) based diagnosis

RT-PCR technology is a variant of PCR, which is generally used in molecular biology to detect RNA transcript levels (Freeman *et al.*, 1999). Unlike PCR, in RT-PCR, the viral RNA template is first used to synthesize a complementary DNA (cDNA) with the help of an enzyme called reverse transcriptase. The synthesized cDNA is then used as a template for exponential amplification using PCR. Presently, this technique is the most sensitive available for RNA detection (Schmittgen *et al.*, 2000). The exponential amplification by RT-PCR allows for a highly sensitive technique in which a very low copy number of RNA molecules can be detected. RT-PCR assay is commonly applied in the diagnosis of majority of the diseases caused by RNA viruses utilizing reverse transcriptase PCR and converting their delicate RNA genome into stable cDNA (Longjam *et al.*, 2011). Due to its simplicity, specificity and sensitivity in amplifying RNA targets, numerous scientists including veterinarians have used RT-PCR as a reliable tool for diagnosis of more complex diseases such as foot and mouth diseases (FMD) and avian flu virus among others (Callens and De Clercq, 1999).

Nested PCR based diagnosis

Nested PCR is a modification of the conventional PCR that is designed to minimize non-specific binding in products as a result of the amplification of unwanted primer binding sites (van Pelt-Verkuil *et al.*, 2008). This technology involves the use of two sets of primers and two sequential PCR reactions. The first set of primers are designed to bind to sequences upstream from the second set of primers and are used in an initial PCR

reaction. The products obtained from the initial PCR reaction are then used as template for the next round of PCR amplification using primers internal to those used for the first round PCR reaction (Hanlon and Nadin-Davis, 2013). The PCR sensitivity and specificity may be enhanced significantly, thus even suboptimal nucleic acid samples, such as those extracted from formalin-fixed, paraffin-embedded tissue can be amplified.

The use of nested PCR in veterinary diagnosis has remarkably improved the detection sensitivity of infectious diseases, as such, the technique has been well-established for the detection of various agents of veterinary interest such as arbovirus, avian leucosis virus (ALV), new castle disease virus (NDV), foot and mouth disease virus (FMDV) and avian influenza virus (AIV) (Dhama *et al.*, 2011).

Quantitative Real-time PCR (qPCR) based diagnosis

Quantitative Real-time PCR (qPCR) is the latest development in the conventional PCR technique which has recently been used in veterinary laboratories for fast detection of infectious disease agents. This technique involves the use of a single-tube, closed assay that significantly reduces the problem of cross-contamination between samples (Salisu *et al.*, 2017). The assay is based on the use of dyes (e.g. SYBR Green I) or fluorescent probes (e.g. TaqMan probe) which allowed for the real-time monitoring of amplified products, abolishing the requirement for post-PCR reaction analysis via electrophoresis (Navarro *et al.*, 2015). Presently, qPCR has found widespread application in veterinary diagnostics due to its increased sensitivity, specificity, rapidness, and ability to provide quantitative data (Loftis and Reeves, 2012). This technology has been extensively adopted for the detection of various pathogens, including bacteria, fungi, protozoa, and RNA and DNA viruses (Rahmat *et al.*, 2014). By using this technology, large number of

veterinary samples can be tested within few hours especially at incidence of disease outbreaks (Picardeau *et al.*, 2014). qPCR has been used excellently for the detection of organisms that are very difficult to cultivate, as well as not-yet-cultivated organisms, viruses, and rickettsial agents (Hoffmann *et al.*, 2009). The technique has also been very suitable for the detection and identification of cultivatable yet slow-growing pathogens, including *Mycobacterium*, *Histoplasma*, and *Brucella* (Loftis and Reeves, 2012).

Multiplex PCR base diagnosis

Multiplex PCR is a commonly used molecular biology method that involves the use of multiple primers which permit amplification of multiple targets within a single PCR reaction (Sokolová, 2017). This technique has proven very useful for the rapid and simultaneous identification of multiple pathogens in a given sample (Zhao *et al.*, 2014). The basic principle of multiplex PCR is similar to normal PCR. However, multiplex PCR technique facilitates more rapid pathogen detection as compared to normal PCR through the concurrent amplification of multiple gene targets (Sokolová, 2017). Generally, multiplex PCR assays are useful for diagnostic purposes, providing the veterinarians with the ability to detect more than one infectious agent in a single assay. This technology has been used successfully for the detection of various pathogenic agent of veterinary significance such as *Campylobacter fetus* from the uncultured samples of aborted bovine Fetuses, *Leptospira*, FMD virus (Ahmed *et al.*, 2012). The technique had also been used efficiently for simultaneous detection of porcine circovirus type 2, classical swine fever virus, porcine parvovirus and porcine reproductive and respiratory syndrome virus in pigs (Jiang *et al.*, 2010). Correspondingly, multiplex PCR has be used efficiently for simultaneous detection of six swine DNA and RNA viruses

and eight swine reproductive and respiratory pathogens (M. Zhang *et al.*, 2015).

DNA microarray based diagnosis

DNA microarray also called DNA chip, gene chip or biochip, is an advanced molecular biology technology which was initially developed for mapping of genes and currently used for the detection of a wide range of pathogens of veterinary importance (Salih *et al.*, 2015). This technology involves the use of solid supports such glass slide or silicon chip or nylon membrane layered with up to hundreds of specific oligonucleotide probes capable of targeting a specific part of a gene sequence.

These probes are chemically synthesized short DNA sequences which range from 25 to 80 bp (Sokolová, 2017). Specific samples of nucleic acid fragments (DNA, mRNA or cDNA) is denatured to produce single-stranded fragments which are then labeled with a fluorescent dye by PCR using universal primers or by multiplex PCR. These fragments are subsequently hybridized to the array by binding to their corresponding oligonucleotide probes (Lee *et al.*, 2015). The end results are acquired by observing the fluorescence signal generated from the probe-sample complex. The fluorescence intensity is proportional to the concentration of each labeled nucleic acid fragment (Salisu *et al.*, 2017). DNA microarrays have been considered as a perfect platform for broad-spectrum microbial scrutiny and regarded as one of the most suitable formats for genomic screening and characterization of pathogen transcriptomic responses (Ricke *et al.*, 2015). This technology has the potential to identify the presence of pathogen of interest at a serotype or subspecies level, or to distinguish the agents that cause similar clinical signs, for instance, vesicular lesions (Mishra *et al.*, 2017). Microarray technology has been well established for detection of various pathogens

such as *Mycoplasma spp.*, including *Mycoplasma mycoides* subsp *mycoides* (Tonelli *et al.*, 2012).

Next generation sequencing (NGS) based diagnosis

The demand for rapid, cheap, and precise DNA sequencing data led to the birth and dominance of a recent generation of sequencing technologies called next-generation (Rizzo and Buck, 2012). This recently developed technology is highly promising as it allows for unbiased and massive parallel sequencing without prior knowledge of the complete DNA content in a sample while retaining the flexibility to allow for targeted sequencing (Van Borm *et al.*, 2015). Various NGS platforms are presently available, with each employing different sequencing chemistries and detection strategies (Linnarsson, 2010). NGS technology is nowadays being used progressively to study the etiology, genomics, evolution, and epidemiology of infectious livestock diseases as well as host-pathogen interactions. These applications have brought a novel insights and demonstrate the potential of this recent technology to directly improve on our understanding and control tactics for livestock diseases (Van Borm *et al.*, 2015). The completion of large animal genomes and documentation of genomic variation have been successfully achieved through NGS platforms (Bai *et al.*, 2012). Livestock genomes which provide an essential source of knowledge for understanding food production and animal interaction with infectious pathogens are now available for bovine, pig, sheep, equine, and avian (Bai *et al.*, 2012). Further efforts in livestock genome sequencing have led to documentation of genomic variation that provides information for the development of genetic markers suitable for animal breeding and genetics, including genes that are linked directly to pathogen resistance and interaction with

microbial communities in poultry (Diaz-Sanchez *et al.*, 2013).

Table 1. Summary of some of the merits and demerits of rapid diagnostic approaches.

Techniques	Qualities	Drawbacks	References
ELISA	<ul style="list-style-type: none"> -More rapid and specific than culture base method -Can be automated to lower the assay time. -Handle large number of samples -Ability to detect bacterial toxins 	<ul style="list-style-type: none"> -Sensitivity is low -Not allow multiplexing Vulnerable to false negative result -Antibody/antigen labelling is required -Requires trained personnel 	(Park <i>et al.</i> , 2014; G. Zhang, 2013)
Biosensor Immunoassay	<ul style="list-style-type: none"> -Improved sensitivity -Automated -Handle large number of samples -Specific serotype detection -Not require labelling 	<ul style="list-style-type: none"> -Low specificity, and chances in cross-reactivity with closely related antigens -Involved many washing steps -High cost may involve 	(Ivnitski <i>et al.</i> , 1999; G. Zhang, 2013)
Conventional PCR	<ul style="list-style-type: none"> -Automated -High sensitivity -High specificity -Produce reliable results 	<ul style="list-style-type: none"> -Non-specific amplification is possible -Influence by PCR inhibitors, -Requires DNA purification -Post PCR analysis is required 	(Garibyan and Avashia, 2013; Sokolová, 2017).
RT-PCR	<ul style="list-style-type: none"> -Highly sensitive -Automated 	<ul style="list-style-type: none"> -Extreme sensitivity could lead to undesirable results 	(Gettemy <i>et al.</i> , 1998).
Nested PCR	<ul style="list-style-type: none"> -Enhanced sensitivity and specificity -Automated -Suitable for suboptimal nucleic acid samples -Minimize non-specific products 	<ul style="list-style-type: none"> -Risk of contamination during the transfer of amplified product into another tube for subsequent amplification 	(Carr <i>et al.</i> , 2010; Thompson <i>et al.</i> , 2013).
Real-time PCR	<ul style="list-style-type: none"> -Sensitivity and specificity are high -Rapid cycling and result reproducibility -Amplification can be monitor in real time -No post-PCR processing of products (gel electrophoresis) 	<ul style="list-style-type: none"> -Difficulty in multiplexing -High cost may involve -Requires experienced personnel -Chances of cross contamination 	(Salisu <i>et al.</i> , 2017; Sokolová, 2017)
Multiplex-PCR	<ul style="list-style-type: none"> -Allowed multiplexing (i.e detection of several pathogens at a time) -High sensitivity and specificity 	<ul style="list-style-type: none"> -Primer design is very crucial -live and dead cells are difficult to distinguish I-influenced by PCR inhibitors 	(Park <i>et al.</i> , 2014).(G. Zhang, 2013)
Microarray	<ul style="list-style-type: none"> -Miniaturization, multiplexing, high-throughput screening -High sensitivity and specificity 	<ul style="list-style-type: none"> -Difficulties in prove designing, High cost, data processing is laborious -live and dead cells are difficult to distinguish 	(Park <i>et al.</i> , 2014; Salisu <i>et al.</i> , 2017)
NGS	<ul style="list-style-type: none"> -No prior sequence information is required -Sensitivity, specificity, accuracy and time-efficiency 	<ul style="list-style-type: none"> -Samples need to be prepared and amplified and data analysis required trained personnel, -Sophisticated devices are needed -Fairly expensive 	(Park <i>et al.</i> , 2014; Salisu <i>et al.</i> , 2017; Schadt <i>et al.</i> , 2010)

Conclusions

For many years, a number of conventional diagnostic approaches have been used for diagnosis of various infectious agents either through culture or antibodies detection, however, these approaches are either not precise, specific or lack an optimum degree of sensitivity which cannot be ignored where herd screening is concerned. Maintenance of good animal health generally requires prompt, accurate and instantaneous disease diagnosis. In this regards, molecular diagnostic methods that hold great promise for improving the speed and accuracy of diagnosis for veterinary pathogens were developed. These technics will enhance the effective control and treatment of infectious livestock diseases.

References

- Ahmed, S. A., Sandai, D. A., Musa, S., Hoe, C. H., Riadzi, M., Lau, K. L., & Tang, T. H. (2012). Rapid diagnosis of leptospirosis by multiplex PCR. *The Malaysian journal of medical sciences: MJMS*, 19(3), 9.
- Bai, Y., Sartor, M., & Cavalcoli, J. (2012). Current status and future perspectives for sequencing livestock genomes. *Journal of animal science and biotechnology*, 3(1), 8.
- Cai, H., Caswell, J., & Prescott, J. (2014). Nonculture molecular techniques for diagnosis of bacterial disease in animals: a diagnostic laboratory perspective. *Veterinary pathology*, 51(2), 341-350.
- Callens, M., & De Clercq, K. (1999). Highly sensitive detection of swine vesicular disease virus based on a single tube RT-PCR system and DIG-ELISA detection. *Journal of virological methods*, 77(1), 87-99.
- Carr, J., Williams, D. G., & Hayden, R. T. (2010). Molecular Detection of Multiple Respiratory Viruses *Molecular Diagnostics* (pp. 289-300): Elsevier.
- Christy, R. J., & Thirunavukkarasu, M. (2006). Emerging importance of animal health economics-a note. *Tamilnadu J Veterinary and Animal Sciences*, 2, 113-117.
- Dhama, K., Rajagunalan, S., Chakraborty, S., Verma, A., Kumar, A., Tiwari, R., . . . Dilbaghi, N. (2011). Molecular diagnosis of animal diseases: The current trends and perspectives. *Pakistan journal of biological Sciences*, 16(20), 1-12.
- Diaz-Sanchez, S., Hanning, I., Pendleton, S., & D'Souza, D. (2013). Next-generation sequencing: the future of molecular genetics in poultry production and food safety. *Poultry Science*, 92(2), 562-572.
- Elmahallawy, E. K., Martínez, A. S., Rodriguez-Granger, J., Hoyos-Mallecot, Y., Agil, A., Mari, J. M. N., & Fernández, J. G. (2014). Diagnosis of leishmaniasis. *The Journal of Infection in Developing Countries*, 8(08), 961-972.
- Freeman, W. M., Walker, S. J., & Vrana, K. E. (1999). Quantitative RT-PCR: pitfalls and potential. *Biotechniques*, 26(1), 112-125.
- Garibyan, L., & Avashia, N. (2013). Research techniques made simple: polymerase chain reaction (PCR). *The Journal of investigative dermatology*, 133(3), e6.
- Gasser, R. B. (2006). Molecular tools—advances, opportunities and prospects. *Veterinary parasitology*, 136(2), 69-89.
- Gettemy, J. M., Ma, B., Alic, M., & Gold, M. H. (1998). Reverse transcription-PCR analysis of the regulation of the manganese peroxidase gene family. *Applied and Environmental Microbiology*, 64(2), 569-574.
- Gibbs, E. (2005). Emerging zoonotic epidemics in the interconnected global community. *The Veterinary Record*, 157(22), 673.
- Hanlon, C. A., & Nadin-Davis, S. A. (2013). Laboratory diagnosis of rabies *Rabies (Third Edition)* (pp. 409-459): Elsevier.
- Hoffmann, B., Beer, M., Reid, S. M., Mertens, P., Oura, C. A., Van Rijn, P. A., . . .

- Alexander, D. J. (2009). A review of RT-PCR technologies used in veterinary virology and disease control: sensitive and specific diagnosis of five livestock diseases notifiable to the World Organisation for Animal Health. *Veterinary microbiology*, 139(1-2), 1-23.
- Ivnitski, D., Abdel-Hamid, I., Atanasov, P., & Wilkins, E. (1999). Biosensors for detection of pathogenic bacteria. *Biosensors and Bioelectronics*, 14(7), 599-624.
- Jiang, Y., Shang, H., Xu, H., Zhu, L., Chen, W., Zhao, L., & Fang, L. (2010). Simultaneous detection of porcine circovirus type 2, classical swine fever virus, porcine parvovirus and porcine reproductive and respiratory syndrome virus in pigs by multiplex polymerase chain reaction. *The Veterinary Journal*, 183(2), 172-175.
- Kuleš, J., Potocnakova, L., Bhide, K., Tomassone, L., Fuehrer, H.-P., Horvatić, A., . . . Mrljak, V. (2017). The Challenges and Advances in Diagnosis of Vector-Borne Diseases: Where Do We Stand? *Vector-Borne and Zoonotic Diseases*, 17(5), 285-296.
- Lee, K.-M., Runyon, M., Herrman, T. J., Phillips, R., & Hsieh, J. (2015). Review of Salmonella detection and identification methods: aspects of rapid emergency response and food safety. *Food Control*, 47, 264-276.
- Lew-Tabor, A. E. (2009). Molecular biotechnology: Applications in Livestock Systems. *biotechnology-Volume II: Fundamentals in Biotechnology*, 2, 168.
- Linnarsson, S. (2010). Recent advances in DNA sequencing methods—general principles of sample preparation. *Experimental cell research*, 316(8), 1339-1343.
- Loftis, A. D., & Reeves, W. K. (2012). Principles of Real-Time PCR. *Veterinary PCR Diagnostics*, 3.
- Longjam, N., Deb, R., Sarmah, A., Tayo, T., Awachat, V., & Saxena, V. (2011). A brief review on diagnosis of foot-and-mouth disease of livestock: conventional to molecular tools. *Veterinary medicine international*, 2011.
- Mishra, C., Mishra, S. P., Sabat, S., Sethy, K., & Behera, K. (2017). Biotechnological tools in disease diagnosis in animal. *IJCS*, 5(6), 611-617.
- Navarro, E., Serrano-Heras, G., Castaño, M., & Solera, J. (2015). Real-time PCR detection chemistry. *Clinica chimica acta*, 439, 231-250.
- Park, S. H., Aydin, M., Khatiwara, A., Dolan, M. C., Gilmore, D. F., Bouldin, J. L., . . . Ricke, S. C. (2014). Current and emerging technologies for rapid detection and characterization of Salmonella in poultry and poultry products. *Food microbiology*, 38, 250-262.
- Pfaffl, M., Vandesompele, J., & Kubista, M. (2009). Real-time PCR: current technology and applications: Caister Academic Press. London, GB.
- Picardeau, M., Bertherat, E., Jancloes, M., Skouloudis, A. N., Durski, K., & Hartskeerl, R. A. (2014). Rapid tests for diagnosis of leptospirosis: current tools and emerging technologies. *Diagnostic microbiology and infectious disease*, 78(1), 1-8.
- Prajapati, B., Gupta, J., Pandey, D., Parmar, G., & Chaudhari, J. (2017). Molecular markers for resistance against infectious diseases of economic importance. *Veterinary world*, 10(1), 112.
- Rahmat, Z., Mahmood, A., Abdullah, K., & Zafar, Y. (2014). Cotton germplasm of Pakistan *World Cotton Germplasm Resources*: InTech.
- Ricke, S. C., Dawoud, T. M., & Kwon, Y. M. (2015). Application of molecular methods for traceability of foodborne pathogens in food safety systems *Food Safety* (pp. 37-63): Elsevier.

- Ridpath, J. F., & Bolin, S. R. (1998). Differentiation of types 1a, 1b and 2 bovine viral diarrhoea virus (BVDV) by PCR. *Molecular and cellular probes*, 12(2), 101-106.
- Rizzo, J. M., & Buck, M. J. (2012). Key principles and clinical applications of “next-generation” DNA sequencing. *Cancer prevention research*, 5(7), 887-900.
- Salih, D., El Hussein, A., & Singla, L. (2015). Diagnostic approaches for tick-borne haemoparasitic diseases in livestock. *Journal of Veterinary Medicine and Animal Health*, 7(2), 45-56.
- Salisu, I. B., Shahid, A. A., Yaqoob, A., Ali, Q., Bajwa, K. S., Rao, A. Q., & Husnain, T. (2017). Molecular Approaches for High Throughput Detection and Quantification of Genetically Modified Crops: A Review. *Frontiers in plant science*, 8, 1670.
- Schadt, E. E., Turner, S., & Kasarskis, A. (2010). A window into third-generation sequencing. *Human molecular genetics*, 19(R2), R227-R240.
- Schmitt, B., & Henderson, L. (2005). Diagnostic tools for animal diseases. *Revue scientifique et technique-Office international des épizooties*, 24(1), 243.
- Schmittgen, T. D., Zakrajsek, B. A., Mills, A. G., Gorn, V., Singer, M. J., & Reed, M. W. (2000). Quantitative reverse transcription-polymerase chain reaction to study mRNA decay: comparison of endpoint and real-time methods. *Analytical biochemistry*, 285(2), 194-204.
- Shojaei, T. R., Tabatabaei, M., Shawky, S., Salleh, M. A. M., & Bald, D. (2015). A review on emerging diagnostic assay for viral detection: the case of avian influenza virus. *Molecular biology reports*, 42(1), 187-199.
- Shyma, K., Gupta, J. P., Singh, V., & Patel, K. (2015). In vitro detection of acaricidal resistance status of Rhipicephalus (Boophilus) microplus against commercial preparation of deltamethrin, flumethrin, and fipronil from North Gujarat, India. *Journal of parasitology research*, 2015.
- Sokolová, I. (2017). Využití non-kultivačních metod ke studiu mikroflóry fermentovaných potravin a nápojů.
- Thompson, A., Bench, S., Carter, B., & Zehr, J. (2013). Coupling FACS and genomic methods for the characterization of uncultivated symbionts *Methods in enzymology* (Vol. 531, pp. 45-60): Elsevier.
- Tomley, F. M., & Shirley, M. W. (2009). Livestock infectious diseases and zoonoses: The Royal Society.
- Tonelli, A., Sacchini, F., Krasteva, I., Zilli, K., Scacchia, M., Beaupaire, C., . . . Pini, A. (2012). One test microbial diagnostic microarray for identification of Mycoplasma mycoides subsp. mycoides and other Mycoplasma species. *Molecular biotechnology*, 52(3), 285-299.
- Tsalik, E. L., Bonomo, R. A., & Fowler Jr, V. G. (2018). New molecular diagnostic approaches to bacterial infections and antibacterial resistance. *Annual review of medicine*, 69, 379-394.
- Van Borm, S., Belák, S., Freimanis, G., Fusaro, A., Granberg, F., Höper, D., . . . Rosseel, T. (2015). Next-generation sequencing in veterinary medicine: how can the massive amount of information arising from high-throughput technologies improve diagnosis, control, and management of infectious diseases? *Veterinary infection biology: molecular diagnostics and high-throughput strategies* (pp. 415-436): Springer.
- van Pelt-Verkuil, E., Van Belkum, A., & Hays, J. P. (2008). *PCR primers*: Springer.
- Zhang, G. (2013). Foodborne pathogenic bacteria detection: an evaluation of current and developing methods. *Meducator*, 1, 24.

- Zhang, M., Xie, Z., Xie, L., Deng, X., Xie, Z., Luo, S., . . . Khan, M. I. (2015). Simultaneous detection of eight swine reproductive and respiratory pathogens using a novel GeXP analyser-based multiplex PCR assay. *Journal of virological methods*, 224, 9-15.
- Zhao, X., Lin, C.-W., Wang, J., & Oh, D. H. (2014). Advances in rapid detection methods for foodborne pathogens. *J. Microbiol. Biotechnol*, 24(3), 297-312.