Monoclonal antibody and its use in the diagnosis of livestock diseases

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ABSTRACT

Since the discovery of hybridoma cells by Kohler and Milstein, the uses of monoclonal antibody (mAb), the protein produced by such cells are in vogue. Such antibodies with single isotype have higher specificity, and the serological tests employed show higher reproducibility compared to those with use of polyclonal antisera. There are several procedures of mAb production which vary considerably but the principle remains the same which states that antigens introduced into animals generally result in the stimulation of lymphocytes, some of which produce antibody of only one type, although the isotype may change. The developments in the field of cell culture and transfection technology have lead to the production of improved qualities of mAbs. In general, monoclonal antibodies are important reagents used in biomedical research, such as, in the field of diagnostics and therapeutics as well as targeted drug delivery systems. They have got importance not only for infectious diseases caused by microbes and parasites, but also for cancer, metabolic and hormonal disorders, in the diagnosis of lymphoid and myeloid malignancies and tissue typing, enzyme linked immunosorbent assay (ELISA) (especially blocking ELISA), radio immunnoassay (RIA), serotyping of pathogens and their immunological intervention with passive antibody, anti-idiotype inhibition or magic bullet therapy with cytotoxic agents coupled with antimouse specific antibody. The application of mAbs in diagnosis of various livestock diseases is an important area of concern as these diseases are a major and increasingly important factor reducing livestock productivity in various parts of the world. In this context, the application of mAbs for diagnosis of important bacterial diseases viz., Anthrax, Brucellosis, Paratuberculosis, Leptospirosis, Listeriosis, Clostridial infections and Mycoplasmosis (CBPP), fungal diseases viz., Zygomycosis, Cryptococcosis, Histoplasmosis and Paracoccidioidomycosis, viral diseases viz., Foot-and-mouth disease (FMD), Infectious bovine rhinotracheitis/Infec-tious pustular vulvovaginitis (IBR/IPV), Rota viral diarrhoea, Blue tongue, Rabies, Classical swine fever and re-emerging viral diseases like Hendra and Nipah viral infections and parasitic diseases viz., dirofilariosis, and Trichinellosis and haemportozoan diseases (including Trypanosomiasis, Leishmaniasis, Anaplasmosis, infections caused by Plasmodium spp. as well as tick borne diseases) have been discussed thoroughly along with the specifications of the diagnostic assays for each disease for the convenience of the diagnosticians, researchers, scientists and students to employ such assays, both in field and laboratories to strengthen the disease control programme.

Keywords: Antibody; Cell Engineering; Diagnosis; Livestock; Microbes; Monoclonal; Parasites

1. INTRODUCTION

In the year 1975, Köhler and Milstein discovered anti-
body producing cells, by fusing mouse myeloma cells with spleen cells of immunized mice. They found the hybridoma cells to have single specificity, characteristic of lymphocytes from immunized mice, but with the myeloma cells’ ability to multiply continuously [1]. The hybridoma cells express the property of specific antibody production specific to lymphocytes and the immortal characters of the myeloma cells that allow it to grow in tissue culture. Interestingly, hybridomas are monoclonal because the cells in culture originate from the division of one cell. The hybridoma can be injected into histocompatible mice to produce ascites cell tumours which become a source of high-titred monoclonal antibody and thereby can be frozen in liquid nitrogen for indefinite periods. When needed, the cells can be thawed and recultured for continued production of the identical specific antibody. Once established, a hybridoma cell line continues to produce identical antibody molecules, enabling us to have a technology for production of an unlimited standardized supply of a high specific antibody that can be used worldwide in antibody-mediated reactions.

We know that the hybridoma growing in tissue culture produces a monoclonal antibody (mAb), but the interesting fact is that the mAb thus produced is chemically, physically, and immunologically a distinct molecule specifically produced by all the cells of a clone. Importantly, a monoclonal antibody is an antibody directed against one antigenic determinant or epitope of an antigen and is a single isotype. By testing a number of different clones it is possible to select those producing mAb of an appropriate specificity and isotype suitable for a particular serological assay. Different animals make different antibodies against each determinant. There is no guarantee whatsoever of reproducibility from animal to animal in polyclonal serum production. If the animals are killed after a production lot, there is a limited supply of reagents as the next animal will produce antibodies of different specificity, affinity, isotype. The homogeneity, reproducibility and permanent availability of monoclonal antibodies are the attributes that are responsible for creating great interest and research fervour in this quickly developing field [2].

2. PRINCIPLES OF PRODUCTION OF MONOCLONAL ANTIBODY

There are several procedures of mAb production and the methods vary considerably [3,4] but the principle remains the same which states that antigens introduced into animals generally result in the stimulation of lymphocytes, some of which produce antibody. Each lymphocyte produces only one type of antibody although the isotype may change and there may be variation in specificity. Thus, a polyclonal antibody response may be similar in specificity and physicochemical characteristics but not identical. On the other hand, lymphoma (myeloma) cells multiply indefinitely in culture but it is rare to find a line that produces antibody of any known specificity. Strains of lymphoma cells can be selected or obtained that produce no immunoglobulin like molecules and which are deficient in hypoxanthine phosphoribosyltransferase and are therefore unable to survive in selective media like hypoxanthine, aminopentrin and thymidine (HAT). The hybrid cells thus generated inherit hypoxanthine phosphoribosyltransferase from the lymphocyte and have got the ability to multiply indefinitely from the lymphoma cell. Some of the hybrid cells will produce antibody of the desired specificity as well as isotype and subsequent cloning and reclone will result in a cell line that produces mAb i.e. hybridoma [1].

3. CELL ENGINEERING FOR MONOCLONAL ANTIBODY PRODUCTION

The development of improved production of mAbs and other recombinant proteins is often associated with progress in cell culture technology and particularly in mammalian cell culture [5]. Other cells commonly used for large-scale production are myeloma cells [6-8], such as SP 2/0, YB 2/0, NS0 and P3X63.Ag8.653 [12]. The production of mAbs in mammalian cells consists of a long process that involves steps of transfection of the gene of interest into the cells, wherein the two elements most widely used as promoters/enhancer are derived from the simian virus 40 (SV40) and cytomegalovirus (CMV). This is followed by the selection of clones and their adaptation to different culture conditions (usually suspension and serum-free medium), culture in bioreactors and scale-up to industrial level. Indeed, due to the high demands for mAbs, a large-scale process needs to be established having levels of production that meet the market needs (several kg/day) [9,10]. For this, the optimization of mAb production is usually performed in bioreactors by testing different bioreactor modes of operation and culture parameters [11].

4. IN GENERAL APPLICATION OF MONOCLONAL ANTIBODIES

In general, monoclonal antibodies (mAbs) are important reagents used in biomedical research, the field in which the use of mAbs has been and will still be necessary for identifying proteins, carbohydrates and nucleic acids, the uses of which have led to the elucidation of many molecules that control cell replication and differentiation. This has advanced our knowledge of the relationship between molecular structure and function, whose application in basic biologic sciences have improved our un-
understanding of the host response to infectious-disease agents and transplanted organs, to toxins produced by infectious agents, to tissues and spontaneously transformed cells (tumors), and to endogenous antigens (involved in autoimmunity), in addition to which, the exquisite specificity of mAb allows them to be used in humans and animals for disease diagnosis and treatment. Thus, in a simplified manner, it can be said that mAbs have tremendous applications in the field of diagnostics and therapeutics as well as targeted drug delivery systems. This is true not only for infectious diseases caused by microbes and parasites, but also for cancer, metabolic and hormonal disorders, in the diagnosis of lymphoid and myeloid malignancies and tissue typing, enzyme linked immunosorbent assay (ELISA), radio immunoscay (RIA), serotyping of pathogens and their immunological intervention with passive antibody, antiidiotype inhibition or magic bullet therapy with cytotoxic agents coupled with anti-mouse specific antibody. Interestingly, the use of mAbs in competitive ELISA, also referred to as blocking ELISA, has come to the forefront as a method to detect the presence of anti-organism antibody. Since introduced by Anderson, the use of MAbs as a method to detect the presence of anti-organism antibody, question always arises about what quantities are used for routine diagnostic procedures and for therapeutic purposes.

Out of all the several applications of mAbs, the authors have found the application of mAbs in livestock diseases diagnosis as an important area of concern as these diseases are a major and increasingly important factor reducing livestock productivity [15,16] in various parts of the world.

5. APPLICATION OF MONOCLONAL ANTIBODY (MABS) TO DIAGNOSE LIVESTOCK DISEASES

Because of their specificity, mAbs have been widely used in the diagnosis of various diseases of livestock that are infectious in nature. Moreover, mAb-based immunodiagnostic assays have several advantages over polyclonally based assays that make the former likely to reduce such variability since they are readily available in unlimited quantities as appropriate and since monoclonal antibodies don’t exhibit much batch-to-batch variability.

5.1. Use of Mabs to Diagnose Microbial Diseases

5.1.1. For Detection of Antigen as Well as Antibodies against Bacterial Diseases of Livestock

Anthrax, a highly infectious and fatal disease of all warm blooded animals and human, is caused by a giant spore-forming rectangular shaped bacterium called Bacillus anthracis [17]. The similarity of endospore surface antigens between bacteria of the Bacillus cereus group complicates the development of selective antibody-based anthrax detection systems and interestingly, the surface of B. anthracis endospores exposes a tetrasaccharide containing the monosaccharide anthrose. Production of anti-tetrasaccharide mAbs and anti-anthrose-rhamnose disaccharide MAbs and testing for their fine specificities in a direct spore ELISA with inactivated spores of a broad spectrum of strains of B. anthracis and related species of the Bacillus genus revealed that although the two sets of mAbs have got different fine specificities, all of them can recognize the tested B. anthracis strains and show only a limited cross-reactivity with two B. cereus strains. The mAbs have been further tested for their ability to be implemented in a highly sensitive and specific bead-based Luminex assay, which detects spores from different strains of B. anthracis and two cross-reactive strains of B. cereus that correlates with the results obtained in direct spore ELISA. The detection limit of the Luminex assay is $10^3$ to $10^7$ spores per ml which is much more sensitive than the corresponding sandwich ELISA, instead of the fact that enzyme assay represents a useful first-line screening tool for the detection of B. anthracis spores [18].

Brucellosis is one of the most extended bacterial
The reactivity of mAb 12G12 has been analyzed in reimmunoblotting or c-ELISAs with specific mAbs [25]. Of the antibody response to outer-membrane protein (OMP) been made to identify the main polypeptide specificities gated with a fluorescent probe. Several attempts have been used from time to time for immunochemical identification of several mAbs have been used from time to time for immunochemical identification of several B. abortus lipopolysaccharide (LPS) epitopes viz., A and M epitopes of smooth strains and R1 and R2 epitopes of rough strains [22]. It is quiet noteworthy that a method which is gaining prominence nowadays is the competitive ELISA (cELISA) [23], wherein, Brucella antigen is immobilised on the plate, following which, the serum under test and a mAb directed against an epitope on the antigen are co-incubated. This anti-Brucella mAb is conjugated to an enzyme and the presence of this particular antibody is detected if it binds to the antigen. This will only occur if there is no antibody in the serum sample which is bound preferentially. Another test in which case mAb find its application is the Particle Concentration Fluorescence Immunoassay (PCFIA) [24], wherein, the mAb is conjugated with a fluorescent probe. Several attempts have been made to identify the main polypeptide specificities of the antibody response to outer-membrane protein (OMP) extracts of B. melitensis of sheep and goat by using either immunoblotting or c-ELISAs with specific mAbs [25]. The reactivity of mAb 12G12 has been analyzed in regard to the main biowars of Brucella species and is found to be strictly directed against the common specific epitope of the Brucella S-LPS, recognizes all of the smooth Brucella strains and biowars except B. suis biowar 2, and a cELISA has been developed with the horseradish peroxidase (HRPO)-conjugated mAbs 12G12 and S-LPS of B. melitensis Rev1 in order to improve the specificity of the serological diagnosis of brucellosis. Apart from that, mAbs in Brucellosis also finds its application in differentiating infected sheep and goat from those vaccinated with Rev. 1 vaccine, directed against the periplasmic protein coding the B. melitensis 16 M bp26 gene [26-28].

Paratuberculosis (Johne’s disease) is a chronic infectious dread disease of domestic animals caused by Mycobacterium avium subsp. paratuberculosis (MAP), and causes huge production losses and has high impact on animal industry [29]. mAbs have been generated against the Ag85a complex of MAP, the sensitivity and specificity of the Ag85a antibody test has been compared with the ELISA and the fecal culture tests for the detection MAP and a serum bank has been established with a view to differentiate MAP-positive and negative cattle.

Leptospirosis is an economically important zoonotic bacterial infection of livestock that causes abortions, stillbirths, infertility along with loss of milk production [30]. Production of a murine mAb, designated as M553 that binds to an epitope on whole cell antigens prepared from Leptospira borgpetersenii serovar hardjo type hardjobovis and Leptospira interrogans serovar hardjo type hardjoprajitno has been reported. Such murine mAb based cELISA assay is found to be advantageous as comparative analysis with microscopic agglutination test (MAT) can also be done [31].

As far as the disease Listeriosis is concerned, colorimetric monoclonal ELISA is intended for the detection of Listeria spp. in dairy products, seafood and meats. However, the test is not confirmatory for L. monocytogenes as the MAbs used in the test may cross react with other Listeria spp [32-34].

Clostridial diseases are caused by bacteria of the genus Clostridium [35] that are widely recognized as enteric pathogens of livestock and wildlife. In spite of the ready availability of inexpensive, usually effective products for immunoprophylaxis, clostridial enteric infections remain a common presentation at Veterinary diagnostic laboratories. mAbs obtained from mouse immunized with Clostridium botulinum type D toxoid have been developed into a sandwich ELISA (sELISA) format that has got the capability to detect type D toxin and types C and D toxin complexes. And Its potential to replace the mouse bioassay as an alternative in vitro assay for the diagnosis of cattle botulism is under examination. The application of this procedure for screening intestinal samples for strains of C. botulinum that produce types C and D toxins from suspect cattle botulism cases would improve the diagnostic rate as well as significantly reduce the number of mice involved in diagnosis [36].

Contagious Bovine Pleuropneumonia (CBPP) is a respiratory infection caused by Mycoplasma mycoides sub species mycoides [37]. Difficulty in isolation requires conjunction with serological testing [38]. Ayling et al. (1998) [39] identified a mAb, M92/20, for use in indirect immunohistochemistry (IHC) for confirmation of suspected CBPP cases and this monoclonal shows no background noise but some cross-reactivity with other Mycoplasmas from the Mycoplasma mycoides cluster is known. Other monoclonals can also be evaluated in this test; in an examination of 11 CBPP affected lungs from Portuguese cattle, IHC has been found to detect all cases which illustrates that M92/20 based IHC is a sensitive
and robust test for CBPP.

5.1.2. For Detection of Antigen as Well as Antibodies against Fungal Diseases of Livestock

The incidence of invasive fungal infections multiplied dramatically in recent decades [40]. Zygomycosis in animals is caused by the fungi of the class Zygomycetes. In most countries, infections are predominantly caused by Aspergillus fumigatus, Candida albicans, Absidia oryzae, and so also Rhizomucor (Muco) pusillus. However, diagnosing these infections in a timely fashion is often very difficult as conventional microbiological and histopathological approaches generally are neither sensitive nor specific. Moreover, such approaches often do not detect invasive fungal infection until late in the course of disease for which there has been considerable interest in developing nonculture approaches to diagnose fungal infections wherein, mAb based diagnosis play an important role, since early diagnosis may guide appropriate treatment and prevent mortality [41]. A number of early studies focused on using cell wall components of fungal species as antigenic markers, among which mannan and galactomannans are useful in the diagnosis of invasive aspergillosis and candidiasis. Early efforts to detect antigenemia were often hampered by the use of insensitive methods with low detection limits [42]. Moreover, fungal mannans or galactomannans may be rapidly removed from circulation by the formation of immune complexes and by receptor-mediated endocytosis by Kupffer's cells in the liver [43], thereby limiting the sensitivity of these diagnostic approaches and hence, attempts have been made toward development of immunoassays based on mAbs with increased specificity and sensitivity. However, much less interest has been shown in diagnosis of other systemic fungal infections.

Invasive aspergillosis is an increasingly recognized condition in immunocompromised hosts and the major problem associated with it is the difficulty in diagnosing this infection. Antibody detection tests are used as an adjunct to microbiological methods for diagnosing invasive aspergillosis that are often negative due to fulminant nature of the disease and/or the poor immunological status of the host [44]. Therefore, the detection of various antigenic markers by mAb based assays for invasive aspergillosis is currently an area of great interest. Stynen and colleagues [45] have introduced a sandwich ELISA, known as Platelia Aspergillus (Bio-Rad, Marnes-la-Coquette, France) which employs rat monoclonal antibody EB-A2 and this test is one of the most sensitive methods currently available to detect galactomannan, whereas, a latex agglutination test (LAT) which employed the same mAb has got a threshold of 15 ng/ml [46]. Furthermore, the sandwich ELISA use to be positive earlier than the LAT and appeared to remain positive after the LAT had become negative. Thus, the development of the Platelia Aspergillus assay represents a marked improvement in the serological diagnosis of aspergillosis. The use of mannan antigenemia (otherwise known as mannanemia) detection for the immunodiagnosis of systemic candidiasis was suggested decades ago by Weiner and Coats-Stephen [46], and it is now one of the most widely studied antigens in candidiasis. The detection of mannanemia in the past has been hampered by the use of insensitive methods that resulted in poor sensitivity and/or specificity [47]. Attempts to improve the immunological detection of mannan involved the use of immune complex dissociation by heating sera before performance of the test along with the use of a more-sensitive test format and mAbs that react with defined epitopes [48]. Several mAbs have been characterized and employed in the immunodiagnostic assays and AF1 is one of them that recognize an oligosaccharide shared by a number of mannoproteins from different pathogenic Candida species [49]. Another mAb, 3H8 (an IgG1), recognized only mannoproteins of high molecular mass present in the Candida albicans cell wall but not those of other Candida species [50]. Other mAbs include EB-CA1, for cases in which different species of the Candida genus share both the EB-CA1 epitope distributed on the mannan and mannoproteins of Candida tropicalis and other species of Candida like Candida glabrata, Candida parapsilosis, and C. krusei [51]. Two assays employing this monoclonal antibody have been marketed as the Pastorex Candida latex agglutination test (Bio-Rad) and the Platelia Candida Antigen test (a double-sandwich enzyme immunoassay) (Bio-Rad). The EIA is more sensitive than the LAT even supposing their specificities to be similar [52]. The etiological diagnosis can also be accomplished by indirect immunofluorescence staining and three-layered indirect enzyme immunochemical techniques using peroxidase anti-peroxidase (PAP) and alkaline phosphatase anti-alkaline phosphatase (APAAP) immunocomplexes with antifungal antibodies viz. a rat IgM monoclonal antibody (EB-A1) against Aspergillus galactomannan [53] and a murine IgG1 mAb (IA-B4) reacting with somatic antigens of A. corymbifera [54]. Monospecific hyper immune rabbit antisera raised against mannan from C. albicans and somatic antigens from A. fumigatus and A. corymbifera can also be applied. The reactivity of antibodies can be assessed on experimentally infected murine and bovine tissues [39]. Fungi that are stained only by the monoclonal rat anti-Aspergillus galactomannan antibody or the monospecific rabbit antibodies to somatic antigens of A. fumigatus are classified as Aspergillus spp., whereas fungi reacting only with the rabbit anti-Candida mannan antibodies are identified as Candida spp. Fungi that are stained with the murine mAb to somatic antigens of A. corymbifera or by the
monospecific rabbit antiserum to somatic antigens of *A. corymbiferu* were classified as zygomycetes in the family *Mucoraceae*.

As far as Cryptococcosis is concerned, the detection of cryptococcal capsular polysaccharide antigen is one of the most valuable rapid serodiagnostic tests for fungi performed on a routine basis. Murex Cryptococcus Test (Murex Diagnostics, Norcross, Ga.) (mouse monoclonal IgM-based latex agglutination assay) effectively eliminates false-positive reactions with rheumatoid factor [55].

Detection of Histoplasmosis (HPA) by RIA in a reference laboratory is an established method for the diagnosis of histoplasmosis and monitoring the response to treatment [56]. However, the limitations of using RIA include the requirement of radioactivity that may not be easily adaptable into a kit form, and the use of polyclonal antisera, which has shown interassay variability [57] as well as cross-reactivity with other dimorphic fungi such as *B. dermatitidis*, *P. brasiliensis*, and *P. marneffei* [58]. Thus, a more specific detection system through the application of mAb is likely to reduce the cross-reactivity with other dimorphic fungi and interassay variation, which led to raising a monoclonal antibody that recognizes a species-specific epitope on a 69- to 70-kDa antigen of histoplasmosis by inhibition ELISA [59]. Recently, Gomez et al. (1997) [60] have developed an inhibition ELISA for the detection of circulating antigen with a mAb P1B directed against an 87-kDa determinant of *Paracoccidioidomycetes brasiliensis* [61], that appears to be a promising method.

Bovine mastitis is the most complex disease condition due to multiple causative agents, poor understanding of the early immune response and complexities associated with mammary epithelial cell damage by both the agents and the host factors and as an important matter of fact, decreased milk production accounts for approximately 70% of the total cost of mastitis [62]. So, diagnosis of mastitis is a centre of attraction for most diagnosticians and clinicians and in this regard, mAb finds its application in direct capture ELISA that can be used to measure elevated polymorphonuclear granulocyte (PMN) antigens using mAb specific for PMN cells along with HRPO conjugated rabbit polyclonal anti-PMN antisera. The test gives an optical density (O.D.) which is found to be useful to predict the cell counts of milk samples [63].

5.1.3. For Detection of Antigen as Well as Antibodies against Viral Diseases of Livestock

Research and development on viruses with respect to diagnosis of virus infections need to be strengthened, an international network of databases of virus infections needs to be instituted and a global network for the diagnosis and containment of viral diseases is advocated [64] for containment and subsequent eradication of the other major infectious diseases of viral origin and in this regard mAb based diagnosis plays a crucial role.

Foot-and-mouth disease (FMD) is a highly contagious disease affecting *Artiodactyla* including cattle worldwide and is included in the notifiable disease list of the World Organization for Animal Health (OIE) [65-67]. It is recognized as a significant epidemic disease threatening the cattle industry since the sixteenth century and till date it is a major global animal health problem [68,69]. In India, annually there are 5000 outbreaks affecting nearly three lakh animals with an economic loss of Rs. 4300 crores. The losses are mainly due to reduction in milk yield, draught power, breeding capabilities etc. and it is a leading cause of loss of livestock economy (direct and indirect losses) due to its endemic nature in India where world’s largest livestock populations exist [70]. So inclusion of mAb based diagnostic methods in the containment programme of FMD is an area of concern. In this regard, per side diagnostic test, especially development of a rapid chromatographic strip test, the lateral flow device (LFD) based on a mAb that reacts against all seven serotypes of FMDV is available. The lateral flow assay (LFA) permits rapid diagnosis, thus allowing time for the early implementation of control measures to reduce the possibility of spread of FMD and the assay has been developed widely to support clinical diagnosis of FMD. Again a simple, rapid, colloidal gold-based immuno-chromatographic strip test based on mAb is also developed for easy clinical testing of serotype A of FMDV in field sites with high sensitivity and specificity [71].

Infectious bovine rhinotracheitis/infectious pustular vulvovaginitis (IBR/IVP) is caused by bovine herpesvirus 1 (BoHV-1). It is also an OIE listed disease affecting domestic and wild cattle. It is one of the most widespread respiratory/reproductive viral diseases of bovines in India [72], where massive cattle and buffalo populations are frequently exposed to IBR. To identify the recovered virus as BoHV-1, the supernatant of the culture where virus isolation has been done, should be neutralised with a monospecific BoHV-1 antiserum or neutralising monoclonal antibody (MAb). An alternative method of virus identification is the direct verification of BoHV-1 antigen in cells around the CPE by an immunofluorescence or immunoperoxidase test [73] with conjugated monospecific antiserum or MAb.

Amongst the various causes of neonatal diarrhoea, group A rotavirus has a world-wide distribution and is considered to be the single most important aetiological agent of acute viral gastroenteritis [74] in various new borne mammalian species that provoke economic losses [75]. In this regard, specific ELISA assays based on mAbs have been developed for identifying the genomic
Blue tongue virus (BTV) is the best studied member of the orbivirus genus in the Reoviridae family. The VP2 protein present in the outer layer of the nucleocapsid of the virus is an important component as far as the sero-grouping of the virus is concerned. This is so because VP2 is involved in virus neutralization. For this purpose, several mAbs have been generated to identify the specific epitopes present on the VP2 protein [77]. Moreover, it is assumed that the VP7 protein, present in small number of viruses is accessible from the outer surface and can be targeted for the detection of BTV, for which gold-labelled mAbs to VP7 are used to give intense labelling with purified BTV core particles. Interestingly, these mAbs do not require any purification too [78].

Rabies, an acute viral encephalomyelitis that may affect all warm blooded animals including cattle, is caused by a lyssavirus. Rabies virus is the most important lyssavirus globally [79]. Although the number of antigenic sites on the rabies virus glycoprotein that have been described regularly increases with time, no attempt has been made earlier to carefully evaluate the relative importance of each of these sites before 1990s. Earlier attempts could identify at least three functionally independent antigenic sites, based on the grouping of the neutralization resistant variant rabies viruses [80]. However, Bennmansour et al. (1991) [81] has provided a more precise description of the antigenicity of the protein in mice of the H-2d haplotype by using more than 250 newly isolated MAbs. Most of the MAbs recognize antigenic sites previously described as II and III and one minor antigenic site separated from site III by three amino acids, including a proline, has been identified as a minor site. Some of the MAbs are also found to neutralize the site III-specific mutant viruses and one of the MAbs, 1D1, has been found to react with sodium dodecyl sulfate-treated glycoprotein in Western blots (immunoblots) under reducing conditions and was therefore probably directed against a linear epitope, which is called G1 (G, Gif). As a general rule, from this study, it has been proposed to reserve the term “antigenic site” (either major or minor) for regions of the protein, defined by several MAbs originating from different fusions and to describe regions of the protein defined by a single mAb as epitopes. Over the years, investigators have found rabies virus in non-hematophagous bats in America, Africa, and Europe. So, in order to identify transmissibility of the virus, analysis of several European rabies virus isolates from various animal species by antinucleocapsid mAbs have been done that have indicated that transmission of the disease from bats to terrestrial animals is unlikely. Moreover, the antigenic profiles of two isolates from European bats have corresponded to that of European bat lyssavirus type 1 (EBL1). Comparisons of different isolates from bats with antinucleocapsid and antiglycoprotein mAbs along with direct sequencing of the polymerase chain reaction amplification product of the N gene have indicated that EBL1 and 2, Duvenhage virus (serotype 4 of lyssavirus) and the European fox rabies virus (serotype 1) are phylogenetically distant [82]. Many of the MAbs (Groups I-IV), having anti-P reactivity and generated by a recombinant rabies virus phosphoprotein fusion product (GST-P) recognizes linear epitopes. They have been judged by their reaction in immunoblots, following which the linear epitope recognized in each case has been mapped by using two series of N- and C-terminally deleted recombinant phosphoproteins. Assessment of the reactivities of representative MAbs to a variety of lyssavirus isolates by an indirect fluorescent antibody test indicates that group I mAbs, which recognize a highly conserved N-terminal epitope, are broadly cross-reactive with all lyssaviruses assayed, while group III mAbs, which react with a site overlapping that of group I mAbs, exhibit variable reactivities. Nevertheless, group IV mAbs react with most isolates of genotypes 1, 6, and 7 only. In contrast, group II mAbs recognize an epitope in a strain-specific manner located within a highly divergent central portion of the protein and these anti-P MAbs are potentially useful tools for lyssavirus discrimination [83].

A mAb based competition-ELISA has been described for the detection of pestivirus antibodies directed against conserved epitopes on the p80 viral protein. This particular method detects increases in serum antibody following experimentally induced infections of pigs and ruminants like cattle and sheep with a wide range of pestiviruses and is highly specific relative to virus neutralization tests. One added advantage of this mAb based assay is that sera from both ruminants and pigs can be assessed without any modification of the test [84]. The use of a panel of three mAbs, either HRPO or FITC conjugated or used in conjunction with an anti-mouse conjugate and specifically detecting all field strains of Classical Swine Fever Virus (CSFV), vaccine strains of CSFV and ruminant pestiviruses, respectively has been reported. A prerequisite is that the MAb against CSFV recognizes all field strains and that the anti-vaccine MAb recognizes all vaccine strains used in the country [85]. No single mAb selectively reacts with all ruminant pestiviruses. This test allows unambiguous differentiation between field and vaccine strains of CSFV on the one hand and between CSFV and other pestiviruses on the other [86].

Hendra virus (HeV) and Nipah virus (NiV) are highly pathogenic paramyxoviruses that have recently emerged from flying fox populations to cause serious disease outbreaks in livestock in India and other Asian countries. Two viral diseases is virus neutralization test (VNT) by
the application of human mAbs that are exceptionally potent in cross-reactive neutralization of the viruses. In this context, neutralizing human mAbs against NiV and HeV have been previously identified by panning a large non-immune antibody library against a soluble form of the HeV attachment-envelope glycoprotein G (sG$_{HeV}$). One of these antibodies, m102, has been found to exhibit the highest level of cross-reactive neutralization of both NiV and HeV G, thus has been affinity maturated by light-chain shuffling combined with random mutagenesis of its heavy-chain variable domain and panning against sG$_{HeV}$. One of the selected antibody Fab clones (m102.4) has got affinity of binding to sG$_{HeV}$ that is equal to or higher than that of the other Fabs. It is converted to IgG1 and tested against infectious NiV and HeV and found to exhibit exceptionally potent and cross-reactive inhibitory activity. The virus-neutralizing activity correlates with the binding affinity of the antibody to sG$_{HeV}$ and sG$_{NiV}$. m102.4 can neutralize NiV better than HeV which suggests that m102.4 has potential as a diagnostic and as a research reagent [88].

The review also requires a special mention of the works based on mAbs that have been carried out as far as the poultry disease diagnosis is concerned, especially in the Indian subcontinent [89-91], classical example being the antigenic characterization and subsequent detection of the minor antigenic differences of Indian isolates and vaccine strains of New Castle disease virus (NDV) [92, 93].

5.2. Use of mAbs to Diagnose Parasitic Diseases

Recent reports have shown that parasite antigens are detectable in the serum of Dirofilaria immitis-infected dogs by counter immuno electrophoresis (CIE). Hybridoma cell lines that produce mAbs specific for these antigens can be obtained by immunizing mice with a partially purified antigen preparation by fusing spleen cells with SP-2 myeloma cells. By screening cell culture supernatants for antibody by ELISA and CIE inhibition, antibodies specific for two epitopes shared by the two major circulating parasite antigens have been identified [94]. For the identification of circulating parasite antigens associated with immune-complex glomerulonephritis in dogs infected with Dirofilaria immitis, mAbs have been generated against adult worms and selected for cloning because of their lack of cross-reactivity with Toxocara canis in indirect immunofluorescence tests. The ability of these mAbs to detect circulating antigens using an antigen-capture ELISA have shown that only few, out of many mAbs, like NAK-1, an IgG2a mAb, is capable of detecting circulating antigens in most of the infected dogs. However, this mAb is highly species-specific in its detection of circulating antigens and detects antigens at the same glomerular sites in which IgG and/or C3 are deposited [95].

mAbs may be a valuable tool in the early diagnosis of Trichinellosis by the detection of specific antigens even in small amounts whenever present in the circulation [96]. In this context, a sandwich ELISA based on IgY (egg yolk immunoglobulin) and mAb against excretory-secretory (ES) antigens (IgM type) of Trichinella spiralis muscle larvae has been developed for detection of circulating antigens (CAg) in serum from mice infected with T. spiralis, involving the use of chicken antibody IgY as a capture antibody and mouse mAb 35B9 as a detecting antibody. This method is able to detect as little as 1 ng/mL of ES antigens added to normal mouse serum that shows high sensitivity of the assay. Moreover, it is also valuable as far as the early diagnosis and evaluation of the efficacy of chemotherapy in trichinellosis is concerned [97] and such IgM mAbs based assay can act as a complementary laboratory test for antibody detection [98].

Trypanosomiasis is the most widely distributed pathogenic mechanically transmitted vector borne haemoprotozoan disease prevalent in all livestock species and is a listed disease of OIE [99]. Species-specific mAbs developed against T. brucei, T. vivax, T. congolense [100] and T. evansi [101,102] allow isolation and purification of defined specific antigens for use in indirect ELISA.

Protozoan parasites of the genus Leishmania cause a spectrum of diseases, varying from self-healing cutaneous leishmaniasis to potentially fatal visceral leishmaniasis (VL) or kala-azar (KA). mAbs specific for selected species complexes of Leishmania have been employed for the characterization of several representative strains of Leishmania [102] including amastigote stage of Leishmania amazonensis. Moreover, mAb has been found to be suitable for isolation and purification of Leishmania antigens. mAb raised against pathogenic promastigotes of Leishmania donovani of Indian origin has been used for immuno-affinity purification of a 78 kDa membrane protein present in both the amastigote and promastigote forms of the parasite and for identification of a 57 kDa antigen of Leishmania infantum [103,104].

The molecular events underlying the commitment of Plasmodium spp. to invade RBC’s are not well understood. However, the interaction of two parasite proteins viz., RON2 and AMA1 is known to be critical for invasion and is essential to trigger junction formation. mAbs specific for the AMA1 pocket block junction formation and the induction of the parasitophorous vacuole. Using these mAbs that bind near the hydrophobic pocket of AMA1, RON2’s binding site on AMA1 can be identified, which helps in parasite identification and subsequent selection of potential vaccine candidate [105].

Babesiosis and anaplasmosis are tick-borne diseases of
cattle caused by the organisms *Babesia bovis*, *B. bigemina* as well as *Anaplasma marginale* [106]. Validation of a *B. bigemina* mAb based competitive ELISA specific for *B. bigemina* has shown to have high sensitivity and specificity [107] and such mAbs are free from cross reaction to that of to *B. bovis*. This assay works well in the absence of any other workable test for *B. bigemina*. mAb also finds its application in a competitive ELISA using recombinant major surface protein 5 (rMSP5) of *A. marginale* which is available in kit form [108].

6. CONCLUSION

Biotechnological advancement has helped in the large scale production of mAbs that forms an integral component of many diagnostic assays viz., immunohistochemistry, immunofluorescence and ELISAs (indirect, competitive or sandwich) that are frequently employed either for detection of the infectious agent or any of its structural component (antigen) or the antibodies generated against the infectious agent. The specifications of the diagnostic assays for each of the many important diseases of livestock that has been discussed in this review will make it convenient for the diagnosticians, researchers, scientists and students to employ such assays, both under field and laboratory conditions to strengthen the disease diagnosis to benefit the disease control programmes and to provide better scope to facilitate eradication of most of them.

REFERENCES


[21] Report of the Scientific Committee on Animal Health and


