Comparative studies on the different laboratory diagnostic methods for Rift Valley Fever virus in domestic animals

By

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SUMMARY

Comparative studies between different serological tests on different species (sheep, goat, cattle and camel) vaccinated by two doses (initial, and booster dose after 30 days) with inactivated vaccine were done to determine the most efficient employed serological test for evaluation of sera for diagnostic purpose and for determination of the humeral – response of different animal species. AGPT gave poor results in all species (5.8-11%), SPA agglutination test gave positive reaction (36.6-43%) more than AGPT and gave the highest percentage of reaction at 120 days, HI test gave positive results (48.3-52%) more than SPA but less than ELISA (57.5-61%) which appeared more than HI, while SNT gave positive reaction (60.8-66%) in parallel correlation with ALISA.

INTRODUCTION

Rift Valley Fever (RVF) is an acute febrile arthropod-borne viral disease. It is a zoonotic disease, highly infectious, and highly fatal among livestock. It is responsible for great losses due to abortions and heavy mortalities in young animals (Easterday 1965, Digoutte and Peters 1989).

The agent of RVF is an enveloped RNA virus belonging to the phlebo genus of the family Bunyaviridae (Bishop and Shope 1979). RVF is firstly recorded in Rift Valley area in Kenya 1931 as described by Daubney et al. (1931), and recorded for the first time in Egypt during 1977-1978 (WHO, 1978; Imam et al., 1978;
Saber et al., 1979 and Meegan, 1979) with about 82 million Egyptian pounds economic losses (General Veterinary Service, 1994).

Reappearance of RVF epidemic during 1981 and 1993 demonstrated that this disease could be extended its geographical boundaries and causes serious human and animal disease as detected for the first time in Saudi Arabia and Yemen (Fagbo, 2002)

Rapid diagnostic tools are needed to detect RVF in both humans and animals in known epidemic regions as well as non endemic areas when the disease introduced for first time and potentially respective extension zones, for this the aim of the present study was :-

1-Evaluation of RVF antibodies in different species of animals after vaccination by inactivated RVF vaccine using different serological tests :
- Serum Neutralization Test (SNT).
- Enzyme Linked Immuno Sorbent Assay (ELISA).
- Haemagglutination –Inhibition (HI) test.
- Slide Protien A (SPA) agglutination test.
- Agar Gel Precipitation Test (AGPT).

2- Comparative studies among different serological tests to choose the best applied technique in diagnosis of RVF virus.

MATERIAL AND METHODS

1-Samples :

Table (1): Number of serum samples collected from different animal species.

<table>
<thead>
<tr>
<th>Animal Species</th>
<th>No of vaccinated animals</th>
<th>No. of days post vaccination</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>15</td>
<td>60</td>
</tr>
<tr>
<td>Sheep</td>
<td>32</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>Goat</td>
<td>40</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Cattle</td>
<td>32</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>Camel</td>
<td>28</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>Total</td>
<td>132</td>
<td>68</td>
<td>68</td>
</tr>
</tbody>
</table>
A total of 404 sera samples were collected from different species of animals (Sheep, Goats, Cattle, and Camels) from different locations in EL-Dakhla, EL wady El-Gadeed governorate. All sera sample were inactivated at 56 ºC for 30 min. and kept at – 20 ºC until applying serological tests.

2. Laboratory Animals:
Two groups of (10 mice) 3-5 days old Swiss Albino suckling mice were used for the titration of RVF virus.

3. Biological Reagents:
The virus was kindly supplied by department of virology, Animal Health Research Institute, Dokki, Egypt. It was propagated in Vero cell line and was titrated according to El Nimr (1980).

-Anti-bovine horse radish peroxidase labeled anti species IgG, was purchased from Sigma Company, used for ELISA diluted immediately in diluting buffer before use (Sigma Company, USA).

-Hyper immune serum was prepared in rabbits and kindly supplied by department of virology, Animal Health Research Institute, Dokki, Giza, Egypt. used as a positive control serum.


-Newborn calf serum free from Mycoplasma and virus antibodies was used at final concentration 10% in growth media for propagation of cell culture.

-Protein A conjugated with peroxidase was purchased from Sigma chemical company, and used for determining RVF antibodies in sera of different animal species by ELISA test.

-Protein A suspension not conjugated was supplied by Reproductive Research Institute El-Haram, used in slide agglutination test for detection of viral antibodies.

-Agar Gel Precipitation Antigen (Reference RVF tissue culture agar gel precipitation antigen) was supplied by Virology department, Animal Health Research Institute, Dokki, Giza, Egypt.

-Tissue culture (Vero cells) were grown and maintained according to (Macpherson and Stockser 1962) and used for RVFV titration. It was supplied by Wister Institute, Philadelphia, USA, and obtained through NAMRO-3 Cairo, Egypt.

4. Media:
Eagle's Minimum Essential Media (MEM) was supplied by Sigma Company, and prepared according to the manufacturer of the company.
5- Evaluation of the immune status of different animal species vaccinated by two doses of inactivated RVF vaccine using different serological tests:
- Serum Neutralization Test (SNT) was carried out according to Edwin and Nathalic (1979).
- Enzyme Linked Immuno Sorbent Assay (ELISA) was carried out according to Voller et al. (1976) and modified by Engvaal and Perlmann (1971).
- Haemagglutination Inhibition (HI) test was carried out by the use of micro – technique.
- Slide SPA agglutination Test was carried out according to Barrow and Felthan (1995) while Agar Gel Precipitation test was done according to Eissa (1984).
- Indirect ELISA technique, according to method described by Voller et al. (1976)

RESULTS
A total of 404 sera samples were collected from different species of animals (Sheep, Goats, Cattle, and Camels) from different locations in EL- Dakhla, EL wady El- Gadeed governorate were illustrated in table (1).

Table (2) : Number of positive serum samples from revaccinated sheep using different serological tests.

<table>
<thead>
<tr>
<th>Date of vaccination</th>
<th>No. of tested samples</th>
<th>AGPT</th>
<th>Slide SPA</th>
<th>HI</th>
<th>ELISA</th>
<th>SNT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
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<td>0</td>
<td>0</td>
<td>6</td>
<td>35.2</td>
<td>10</td>
</tr>
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<td></td>
<td>13</td>
<td>76.4</td>
<td>14</td>
<td>82.4</td>
<td></td>
</tr>
<tr>
<td>Booster dose after 30 days</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>17</td>
<td>5</td>
<td>29.4</td>
<td>10</td>
<td>58.8</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15</td>
<td>88.2</td>
<td>15</td>
<td>88.2</td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>17</td>
<td>2</td>
<td>11.8</td>
<td>11</td>
<td>64.7</td>
<td>11</td>
</tr>
<tr>
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<td></td>
<td>12</td>
<td>70.5</td>
<td>13</td>
<td>76.4</td>
<td></td>
</tr>
<tr>
<td>180</td>
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<td>0</td>
<td>6</td>
<td>35.3</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>58.8</td>
<td>11</td>
<td>64.7</td>
<td></td>
</tr>
<tr>
<td>Total No. of tested samples</td>
<td>100</td>
<td>7</td>
<td>42</td>
<td>42</td>
<td>52</td>
<td>62</td>
</tr>
</tbody>
</table>

*SPA = (Staphylococcus protein A). Negative means sandy like appearance.
* Positive HI =1/8
* Positive ELISA ≥ 0.3 OD
* Positive SNT = 1/40
Table (3): Number of positive serum samples from revaccinated goats using different serological tests.

<table>
<thead>
<tr>
<th>Date of vaccination</th>
<th>No. of tested samples</th>
<th>AGPT</th>
<th>Slide SPA</th>
<th>HI</th>
<th>ELISA</th>
<th>SNT</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>40</td>
<td>0</td>
<td>0</td>
<td>11</td>
<td>14</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>15</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>7</td>
<td>10</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>Booster dose after 30 days</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>20</td>
<td>4</td>
<td>20</td>
<td>8</td>
<td>13</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>120</td>
<td>20</td>
<td>2</td>
<td>10</td>
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<td>%</td>
<td>%</td>
<td>%</td>
</tr>
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<td>180</td>
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<td>5</td>
<td>7</td>
<td>9</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>Total No. of tested samples</td>
<td>120</td>
<td>7</td>
<td>5.8</td>
<td>44</td>
<td>36.6</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td></td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
</tbody>
</table>

Table (4): Number of positive serum samples from revaccinated cattle using different serological tests.

<table>
<thead>
<tr>
<th>Date of vaccination</th>
<th>No. of tested samples</th>
<th>AGPT</th>
<th>Slide SPA</th>
<th>HI</th>
<th>ELISA</th>
<th>SNT</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>32</td>
<td>0</td>
<td>0</td>
<td>9</td>
<td>10</td>
<td>12</td>
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<tr>
<td></td>
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<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
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<td>13</td>
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<td>4</td>
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<td>9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>Booster dose after 30 days</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>60</td>
<td>13</td>
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<td>30.7</td>
<td>7</td>
<td>53.7</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>120</td>
<td>13</td>
<td>1</td>
<td>7.7</td>
<td>8</td>
<td>61.5</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>180</td>
<td>13</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>38.4</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>Total No. of tested samples</td>
<td>84</td>
<td>5</td>
<td>5.9</td>
<td>35</td>
<td>41.9</td>
<td>41</td>
</tr>
<tr>
<td></td>
<td></td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
</tbody>
</table>

* SPA = (Staphylococcus protein A). Negative means sandy like appearance.
* Positive HI = 1/8
* Positive ELISA ≥ 0.3 OD
* Positive SNT = 1/40
Table (5): Number of positive serum samples from revaccinated camel using different serological tests.

<table>
<thead>
<tr>
<th>Date of vaccination</th>
<th>No. of tested samples</th>
<th>AGPT No.</th>
<th>AGPT %</th>
<th>Slide SPA No.</th>
<th>Slide SPA %</th>
<th>HI No.</th>
<th>HI %</th>
<th>ELISA No.</th>
<th>ELISA %</th>
<th>SNT No.</th>
<th>SNT %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>28</td>
<td>0</td>
<td>0</td>
<td>8</td>
<td>28.6</td>
<td>10</td>
<td>35.7</td>
<td>11</td>
<td>39.3</td>
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<td>42.8</td>
</tr>
<tr>
<td>15</td>
<td>18</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>33.3</td>
<td>7</td>
<td>38.9</td>
<td>9</td>
<td>50</td>
<td>10</td>
<td>55.5</td>
</tr>
<tr>
<td>Booster dose after 30 days</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
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<td>18</td>
<td>7</td>
<td>38.8</td>
<td>10</td>
<td>55.5</td>
<td>13</td>
<td>74.2</td>
<td>15</td>
<td>83.3</td>
<td>16</td>
<td>88.8</td>
</tr>
<tr>
<td>120</td>
<td>18</td>
<td>3</td>
<td>16.6</td>
<td>12</td>
<td>66.7</td>
<td>12</td>
<td>66.7</td>
<td>14</td>
<td>77.8</td>
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<td>77.8</td>
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<td>180</td>
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<td>5.5</td>
<td>7</td>
<td>38.9</td>
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<td>55.5</td>
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<td>61.1</td>
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<td>66.7</td>
</tr>
<tr>
<td>Total No. of tested samples</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>43</td>
<td>52</td>
<td>52</td>
<td>60</td>
<td>60</td>
<td>64</td>
<td>64</td>
<td></td>
</tr>
</tbody>
</table>

Table (6): Result of comparative studies among different serological techniques of vaccinated animal species.

<table>
<thead>
<tr>
<th>Animal Species</th>
<th>No. of tested serum samples</th>
<th>AGPT No.</th>
<th>AGPT %</th>
<th>Slide SPA No.</th>
<th>Slide SPA %</th>
<th>HI No.</th>
<th>HI %</th>
<th>ELISA No.</th>
<th>ELISA %</th>
<th>SNT No.</th>
<th>SNT %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sheep</td>
<td>100</td>
<td>7</td>
<td>7</td>
<td>42</td>
<td>42</td>
<td>52</td>
<td>52</td>
<td>60</td>
<td>60</td>
<td>66</td>
<td>66</td>
</tr>
<tr>
<td>Goats</td>
<td>120</td>
<td>8</td>
<td>5.8</td>
<td>44</td>
<td>36.6</td>
<td>58</td>
<td>48.3</td>
<td>64</td>
<td>57.5</td>
<td>73</td>
<td>60.8</td>
</tr>
<tr>
<td>Cattle</td>
<td>84</td>
<td>5</td>
<td>5.9</td>
<td>35</td>
<td>41.9</td>
<td>41</td>
<td>48.8</td>
<td>49</td>
<td>58.3</td>
<td>51</td>
<td>61.9</td>
</tr>
<tr>
<td>Camel</td>
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<td>43</td>
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<td>52</td>
<td>52</td>
<td>61</td>
<td>61</td>
<td>64</td>
<td>64</td>
</tr>
</tbody>
</table>

* SPA = (Staphylococcus protein A). Negative means sandy like appearance.
* Positive HI = 1/8
* Positive ELISA ≥ 0.3 OD
* Positive SNT = 1/40
DISCUSSION

 Rift valley fever (RVF) is an economically important arthropod-borne virus disease in Africa, primarily affecting sheep, cattle, and goats. In these domestic animals the disease causes high mortality in lambs and calves and abortion in pregnant animals (Peters and Meegan, 1981 and Swanepoel and Coetzer, 1994).

Several methods in comparison were used for determination of antibodies to RVF virus such as: Agar Gel Precipitation Test (AGPT), Staphylococcus Protein A (SPA) agglutination test, Haemagglutination Inhibition (HI) test, Enzyme Linked Immunosorbent Assay (ELISA) test and Serum Neutralization Test (SNT).

Our results in Table (2) denote that the highest positive percentage of vaccinated sheep sera were detected by ELISA and SNT (88.2%) after booster dose at 60 days post-vaccination, this agrees with Taha et al. (1994) followed by HI, SPA and AGPT, while serum neutralizing antibodies persist for long time reached to 180 days reached (64.7%), this finding is in agreement with the results obtained by Harrington et al. (1980) who found that serum neutralizing antibodies induced by RVF inactivated vaccine persisted for at least 7 months, but the sensitivity of AGPT raised after booster dose and gave positive reaction which reached (29.4%), this result may due to the antibody level sufficiently high to positive reaction after receiving booster dose this agrees with Ayoub and Allam (1981) and Abdel Ghaffar et al. (1981). They stated that the AGPT reaction only as a result of recent infection or give positive reaction after receiving booster dose, then decreased to give negative reaction at 180 days post vaccination compared with SPA and HI when gave positive reaction but less than ELISA and SNT.

In Table (3) the results of positive percentage of vaccinated goats serum samples revealed the variation between different antibodies detected, the lowest percentage of antibody determined by AGPT (5-20%) and the highest positive percentage was detected by ELISA (37.5 - 80%) and SNT (37.5 - 85%) for this the ELISA and SNT are more sensitive than SPA (27.5 - 55%) and HI (35 - 65%), this agrees with Niklasson et al. (1984) who proved that ELISA test is more sensitive than HI and El-Shinnawy et al. (1987) who determined RVF antibody in goats vaccinated by inactivated vaccine by SNT and HI.

The results in Table (4) indicated that the highest percentage
by SNT was at 60 days post vacci-
nation of cattle (84.6%) and after
booster dose where the level of an-
tibodies reached its peak then de-
creased gradually which persisted
for 180 days post vaccination
(61.5%) as detected by SNT, these
results agree with El Nimr et al.
(1979) and Eissa (1995) who re-
ported that the duration of immune
response may be 6-8 months when
determined the different antibody
level using HI and SNT tests.

Table (5) showed the results
of immune response of camel vac-
cinated with two doses of inacti-
vated vaccine was detected by dif-
ferent serological tests which re-
vealed that the camel had good im-
munogenic status, in which the
level of antibodies was in rise and
become higher after booster dose
(38.8% by AGPT, 55.5% by SPA,
74.2% by HI, 83.3% by ELISA and
88.8% by SNT) these results agree
with Mona (2000). She deter-
mined the antibody response of
vaccinated camel sera (86.3%) us-
ing SNT.

Table (6) revealed that the
AGPT gave poor result compared
with other serological tests per-
formed in all serum samples, this
finding is in agreement with
(WHO 1982) who proved that the
AGPT is considered very specific
but not very sensitive. Moreover
the duration of persistence of the
AGP antibodies has not been as
yet determined. However, despite
its shortcomings the AGPT is sim-
ple to conduct, reliable when posi-
tive, practical and requiring no so-
phisticated equipment, it has also
been satisfactorily applied for di-
agnosis of RVF (Ayoub and Al-
lam, 1981). On the other hand the
SPA agglutinated antibodies were
showed the highest percentage in
camel. The advantages of this test
is rapidly, sensitively, easily per-
formed and screening large quanti-
ties of serum samples at short time
(Furui 1986) who used this tech-
nique for surveying of some dis-
eases. The HI test proved to be
more sensitive than AGPT and
SPA agglutination test but less
pecific than ELISA, the non
pecificity of HI is due to the pres-
ence of cross reaction between
members of phlebo viruses (Nawal
1984 and Scott et al. 1986) but the
results of ELISA test revealed the
antibody response of animal spe-
cies was higher figure of sensitiv-
ity than AGPT, SPA and HI, this
agrees with result obtained by Nik-
lassen et al. (1984) and Taha et al
(2002) who proved that the ELISA
test is more sensitive than HI, the
result of ELISA was in parallel
correlation with SNT which has
agreement to result of Lily et al.
(1999).

Our results demonstrate the
SNT gave higher results in all spe-
cies than other serological tests,
except ELISA sensitivity and
specificity but it requires cell cultures as animal facilities. Furthermore work with live RVF outside endemic regions requires special containment laboratories.

This study concluded that SNT is considered as high specific and sensitive but requires cell culture and animal facilities that can be only performed with live virus with special containment to laboratories, furthermore it needs long time to be performed, so that the ELISA is considered as sensitive and specific test for detecting different antibodies which gave result in parallel with SNT, also ELISA used safe, robust and accurate diagnostic tool in disease surveillance and control programmers, import / export veterinary certification, and at the same time boosting is important to improve the immune system of the animals and could be used as monitoring methods for vaccination programmers.

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دراسات مقارنة لطريقة التشخيص المعمول لفيروس مرض اليرف فالي في حيوانات المزرعة

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المملص العربي

قد أجريت هذه الدراسة للمقارنة بين الاختبارات السيرولوجية المختلفة لمعرفة مدى
حساسية ودقة فاعلية كل اختبار وإختيار الأمثل منها لاستخدامه في التشخيص
ومعرفة الحالة المناعية للحيوانات المختلفة.

وهذه الدراسة أجريت على مجموعة مختلفة من الحيوانات المقدمة لإصابتها بالمرض
وهي (الأغنام - الماعز - الأبقار - الحمار) التي تم تصميمها بجرعتين (أولية وأخرى
منشطة) بعد 20 يوما.

بدأ في الأنسج المناعية المختلفة في مختلف الدراسات بالترسب في الأجسام
وأعلى نسبة من سبعة (6 5 -11%) في كل الحيوانات أما اختبار التجمع البطني
slide (SPA) فعلى الشريحة باستخدام بروتين لمكور الملكيين (3,0-6,0)على
3,0% أعلى من الترسب في الأناج حيث أعلى نسبة التفاعل عند 120 يوما.

وكانت النتائج الإيجابية لإختبار مائع التلزان HI (6,0 - 5 8) أكثر من نتائج اختبار
التجمع البطني ولكن أقل من نتائج اختبار الأليزيا (ELISA) التي ظهرت (5,0 - 11)
أقل من نتائج مائع التلزان بينما أُعطيت اختبار السيرم المتماثل (SNT) (10,0 -
16) متوازية مع اختبار الأليزيا.

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